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Studies of Patients with Hyperglyceridemia

LAURANCE W. KINSELL, M.D.,* GEORGE D. MICHAELS, PH.D.,† GEOFFREY WALKER, M.D.,‡ STANFORD SPLITTER, M.D.§ AND ROBERT E. VISINTINE, M.D.,|| WITH THE TECHNICAL ASSISTANCE OF MARJORIE COELHO, JOYCE CONKLIN, GEORGE FUKAYAMA, FLORENCE OLSON AND SADIE SMYRL

THE SYNDROMES which are characterized by elevation of plasma lipids in human subjects fall into three broad categories; namely, those with predominant elevation of cholesterol with little or no increase in the level of glycerides, those with predominant elevation of phospholipids and free cholesterol but with little or no elevation of glyceride and those with marked elevation of glyceride and with variable degrees of elevation of free and esterified cholesterol and phospholipids. Most of the published material dealing with the latter group has tended to assume that the hyperglyceridemias represent a single entity and that the major metabolic defect is that of impaired removal of glycerides from the circulating plasma.

This paper is concerned with studies of a small group of persons with hyperglyceridemia. Examination of the plasma of all of these patients under conditions of ordinary dietary intake shows moderate or extreme turbidity of the fasting plasma. In our study of these patients, it was postulated that the hyperglyceridemia which is responsible for the turbidity may be attributed to one or more of the following abnormalities: (1) excessive rates of absorption of fat from the intestinal tract, (2) impaired removal of glyceride from the plasma because of the formation of an abnormal type of chylomicron and/or other lipoprotein, (3) impaired removal as a result of abnormal or deficient enzymatic activity within the plasma or intracellularly, (4) excessive mobilization from depots of fat, or (5) disturbance of "lipostatic control." Some of these possibilities have been examined partially.

MATERIALS AND METHODS

The subjects studied included four grossly hyperglyceridemic patients and two persons with no hyperglyceridemia, namely, M. Mor., who had epilepsy, and S. Gre., who had hyperthyroidism. The results of analyses of lipids in plasma from these subjects are given in Table I.

Total lipids were determined by the method of Bragdon,[1] total glycerides by the method of Carlson and Wadström,[2] cholesterol by the method of Michaels et al.,[3] phospholipids

From the Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, California.

* Director; † Senior Research Biochemist; ‡ Nutrition Foundation Research Fellow; § Research Associate; || Research Fellow.

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TABLE I

Plasma Lipid Values and Ratios for Patients Studied

Patient and Diagnosis	Total Lipids (mg./100 ml.)	Total Glyceride (mg./100 ml.)	Cholesterol (mg./100 ml.)			Phospholipids (mg./100 ml.)	Free Fatty Acid (mEq./L.)	T.C.:P.L.	E.C.:F.C.	T.G.:T.L. (per cent)
			Total	Ester	Free					
Nonhyperglyceridemia										
M. Mor. (epilepsy	744	65	265	182	83	250	0.83	1.1	2.9	9
S. Gre. (hyperthyroidism)	556	54	147	102	45	175	1.18	0.8	2.3	10
Hyperglyceridemia										
G. Har.	1,338	475	208	144	64	250	2.46*	0.8	2.3	35
A. Fon.	1,781	788	166	84	82	270	0.50	0.6	1.0	43
E. Dix.	2,475	544	792	566	226	570	6.14*	1.4	2.5	22
M. Per.	6,275	2,925	596	318	278	850	2.79†	0.70	1.2	47

NOTE: T.C. = total cholesterol, P.L. = phospholipid, E.C. = ester cholesterol, F.C. = free cholesterol, T.G. = total glyceride and T.L. = total lipids.

* Receiving subcutaneous injections of heparin daily during this study.

† Levels of free fatty acid in excess of 2.0 mEq. per L. more than 90 per cent of the time.

by the method of Youngburg and Youngburg[4] and free fatty acids by the method of Grossman et al.[5]

Carbon[14] carboxyl-labeled tripalmitin was administered intravenously in a cottonseed oil emulsion (10 μc. in 50 ml. of a 10 per cent emulsion). The emulsion was prepared in the manner described by Singleton and his associates.[6,7]

The initial samples of blood were obtained following an overnight fast and prior to administration of the tripalmitin. The samples were removed from the patients at frequent intervals during the first hour after administration, at hourly intervals for the next eight hours and at daily intervals for a period of seven days. No food or liquid other than black coffee and dilute lemon juice in water sweetened with saccharine were permitted during the eight hour period following administration of the emulsion.

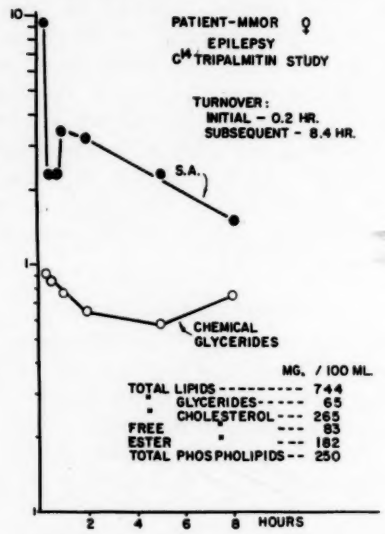

FIG. 1. Depicted are the specific activity and the levels of glycerides in plasma following infusion of C^{14}-tripalmitin to a patient with no hyperglyceridemia. (S. A. = specific activity in all figures.)

After extraction of the lipids from plasma in hot acetone alcohol and re-extraction with petroleum ether, fractionation into total glycerides, cholesterol esters and total phospholipids was carried out by silicic acid column chromatography; free fatty acids were separated by chemical means. Isotopic titer of these fractions was determined in a Packard Automatic Tri-Carb Liquid Scintillation Spectrometer after suitable preparation. (These methods are described in detail elsewhere.[8]) Only the findings concerning glyceride and to a lesser degree the free fatty acids are considered in this paper.

EXPERIMENTAL FINDINGS

Nonhyperglyceridemic Patients

Depicted in Figure 1 are the findings for the patient M. Mor., a woman forty-five years of age, who had had epilepsy for a long period of time.

The findings for the patient S. Gre., a woman

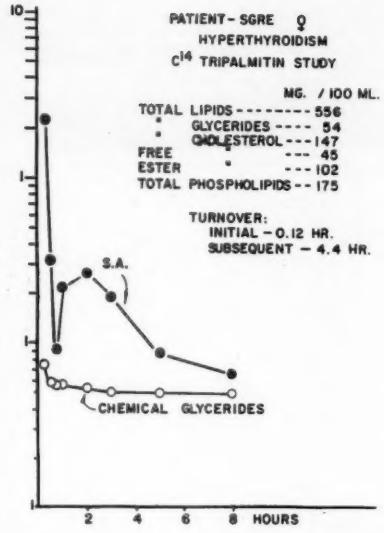

FIG. 2. Specific activity and levels of glycerides in plasma, following infusion of C^{14}-tripalmitin to a patient with hyperthyroidism, are shown. Note the similarities between these findings and those for the patient M. Mor. (Fig. 1) and the dissimilarities when compared to those for other patients.

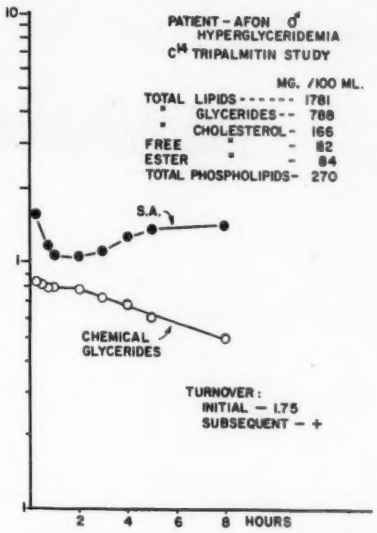

FIG. 3. The results of the C^{14}-tripalmitin study of a patient with hyperglyceridemia are shown. During the period of fast, the levels of glycerides in plasma fell at the rate of nearly 50 mg. per 100 ml. per hour. Secondary rise in specific activity was probably attributable in part to re-entry of labeled glyceride from the liver, with no mobilization of unlabeled fat from depot.

thirty-two years of age with active hyperthyroidism, are illustrated in Figure 2. Assuming that the findings for these patients are reasonably characteristic of nonhyperglyceridemic patients, the following seem apparent: (1) With reference to the chemical glycerides, some homeostatic mechanism appears to be operating to prevent the level of glycerides in plasma from falling below about 50 mg. per 100 ml. Possibly this is, in part, attributable to a specific hormonal factor. (2) Concentration of isotope in the glyceride fraction decreases sharply during the first thirty to forty-five minutes, increases during the next one to two hours and then decreases more or less exponentially during the subsequent eight hours. In both the initial and the latter periods of decline the turnover rate in the hyperthyroid patient (S. Gre.) was more rapid than in M. Mor.

Hyperglyceridemic Patients

Given in Figure 3 are the findings for the patient A. Fon., a thirty year old man. A routine blood test performed when this patient was twenty-six years of age, revealed creamy serum. Two years later he experienced an episode of pain in his chest which radiated down the left arm; two years after this episode he had a sudden attack of dizziness which persisted for several hours. He states that on more than one occasion he has had reddish skin eruptions, particularly in the region of the knees. These have not been noted during the time we have observed him. The results of electrocardiographic examination of this patient were within the normal range as were those of the glucose tolerance and other standard laboratory tests.

Analyses of samples of plasma from this patient were made when he was initially admitted to the hospital in December 1959. The total content of lipids was in excess of 4,000 mg. per 100 ml., the total content of cholesterol was 396 mg. per 100 ml. with a decreased ester:free cholesterol ratio, the content of phospholipids was 365 mg. per 100 ml. and that of free fatty acids was 0.78 mEq. per L.

The patient was fed a diet high in carbo-

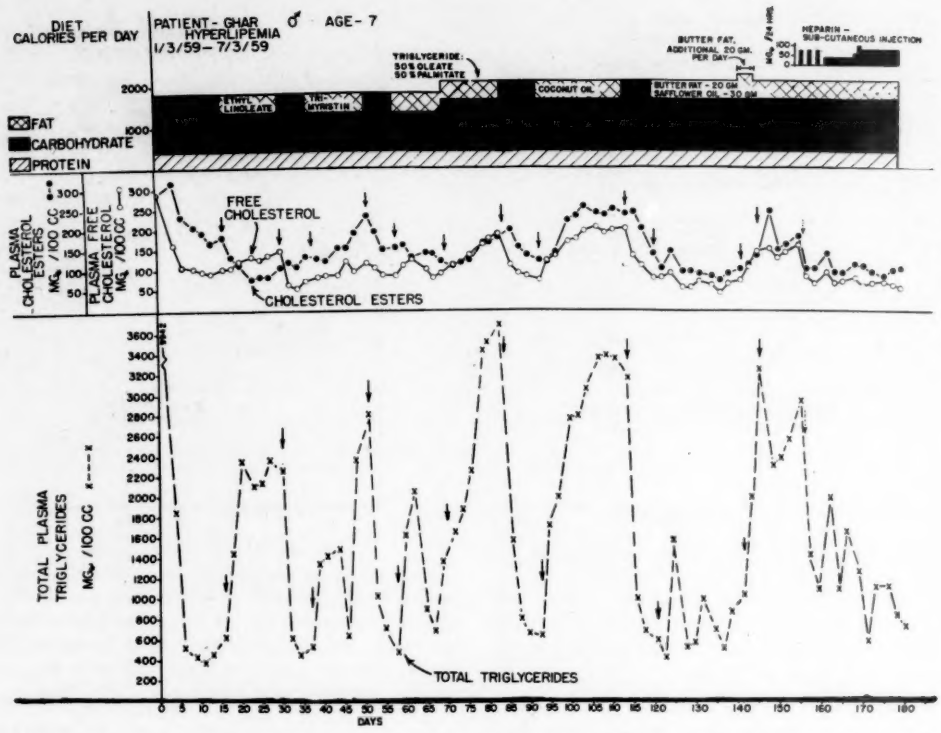

FIG. 4. The results of a long term metabolic study, fat versus no fat, of patient G. Har. are depicted All fats caused a major rise in the levels of glycerides in plasma. A fat-free diet was associated with a rapid fall in the levels of glycerides toward (but not to) normal.

hydrate which contained 30 gm. of predominantly unsaturated fat. The plasma lipids decreased to the following low levels: total lipids, 625 mg. per 100 ml.; total cholesterol, 94 mg. per 100 ml. with a normal ester:free cholesterol ratio; and phospholipids, 145 mg. per 100 ml.

Subsequently, the patient was given a 2,600 calorie diet containing 127 gm. of protein, 60 gm. of predominantly unsaturated fat and 380 gm. of carbohydrate. Determinations of plasma lipids made during this period were as follows: total lipids, 1,778 mg. per 100 ml.; total cholesterol, 124 mg. per 100 ml. with a low ester:free cholesterol ratio; and phospholipids, 213 mg. per 100 ml.

During the administration of the latter diet, the patient received intravenously (over a period of five minutes) 10 μc. of C^{14}-labeled tripalmitin in 50 ml. of 10 per cent cottonseed oil emulsion. The results of this study are shown in Figure 3. Observations made during this study are as follows: (1) During the six hour span from the second to the eighth hour under fasting conditions, the level of glycerides in plasma fell from 788 to 500 mg. per 100 ml., a clearance rate of 48 mg. per 100 ml. per hour. (2) There was an initial rapid decrease in specific activity followed by a plateau and then a significant rise during the period from two to eight hours. (3) The turnover time during the initial decrease was much slower than in the nonhyperglyceridemic subjects previously described.

Patient G. Har., a seven year old boy, was first seen in the emergency ward for severe abdominal pains and the general findings were compatible with the diagnosis of acute pancreatitis. His blood was found to be grossly lipemic. On a program of intravenous infusion of 5 per cent glucose and subsequent withholding of all oral administration of fatty food, his abdominal

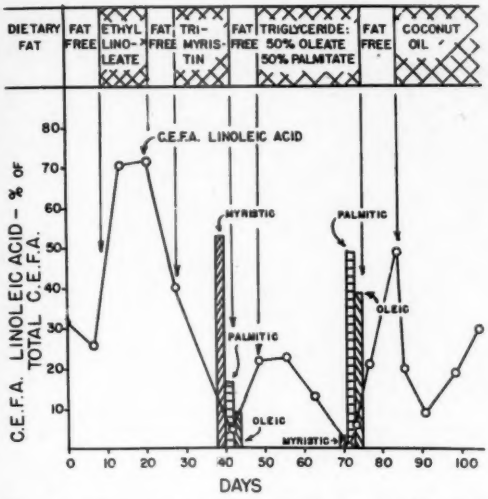

FIG. 5. Illustrated are the effects of specific fats on cholesterol ester fatty acid composition (C.E.F.A.) in patient G. Har. In nonhyperglyceridemic patients, the fatty acid composition of the cholesterol esters is affected slowly, and only to a moderate degree, by the predominant dietary fatty acid. In this patient, the normally high cholesteryl linoleate was almost completely replaced by the predominant dietary fatty acids.

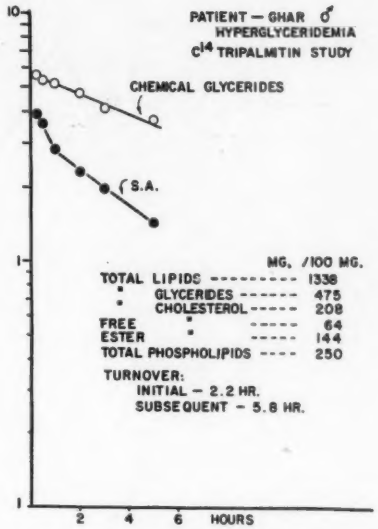

FIG. 6. The results of the C^{14}-tripalmitin study of patient G. Har. are shown. Fall in specific activity appeared to be attributable to continued removal of labeled glyceride at a rate greater than that for the unlabeled material, i.e., there was little or no evidence of mobilization of depot fat in this patient.

symptoms rapidly subsided and after four days his plasma was minimally lipemic. At the time of admission he had widespread skin lesions compatible with the diagnosis of eruptive xanthomas. The following results were obtained several hours after initiation of the glucose infusions when the abdominal pains had lessened: total lipids, 3,375 mg. per 100 ml.; ester cholesterol, 132 mg. per 100 ml.; free cholesterol, 148 mg. per 100 ml.; and total phospholipids, 295 mg. per 100 ml.

He was hospitalized for a six month period from January to July 1959, in the metabolic ward, during which time his response to a variety of dietary programs was determined.[9] Some of the data are provided in Figure 4. It is apparent that the glycerides in plasma were markedly elevated whenever his diet included a significant amount of any type of fat, and that they decreased rapidly when he was placed on a fat-free regimen. The content of glycerides was greater when the fat used was saturated rather than polyunsaturated. In addition, there appeared to be an impressive decrease in the amount of glycerides in plasma when the patient was given heparin. This was subsequently confirmed by more extensive study.

Because the levels of lipids in plasma were somewhat lower when the patient was maintained on such a regimen, daily subcutaneous administration of heparin was incorporated into the program which has been carried out for several months. Unexplained at the present time is the observation that the level of glycerides remained relatively low during an initial period of ingestion of a combination of butter fat and safflower oil.

Also of interest is the pattern of responses of the level of cholesterol ester fatty acids to intake of specific fats (Fig. 5). The cholesterol ester fatty acids promptly reflected the predominant dietary fat. Such reactions have not been observed in nonhyperglyceridemic patients studied. Similar studies of other hyperglyceridemic patients have not yet been carried out.

The results obtained for G. Har. after the administration of 25 ml. of 10 per cent cottonseed oil emulsion containing 5 μc. of C^{14}-tripalmitin are depicted in Figure 6. An eight

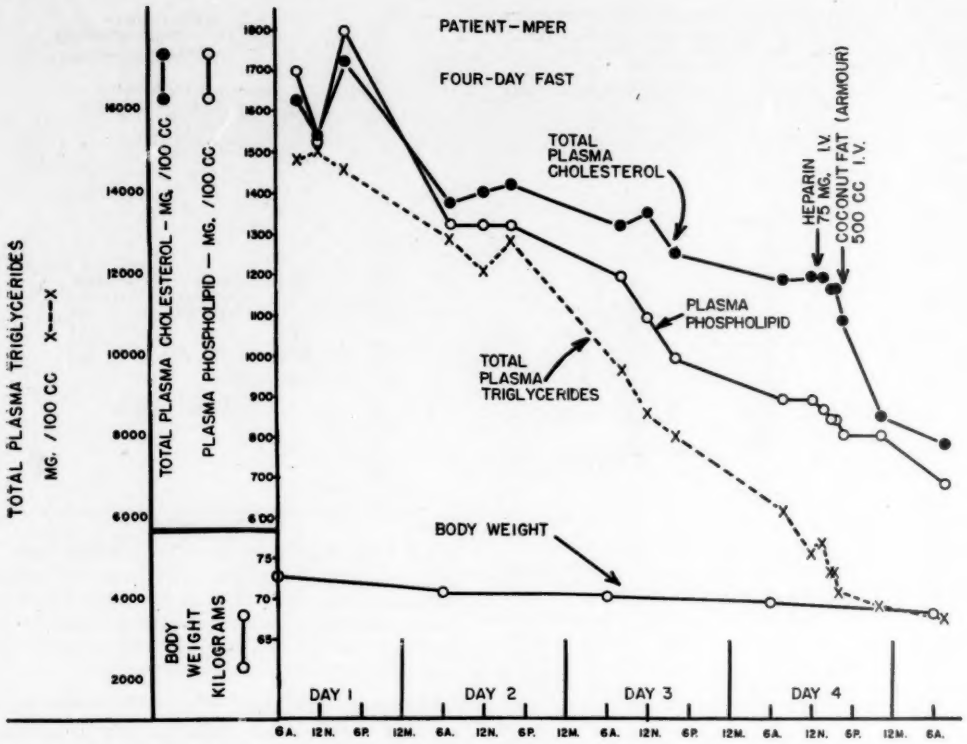

FIG. 7. Complete fasting over a four day period by patient M. Per. (extreme hyperglyceridemia with hyperglycemia) was associated with a rapid decrease in the levels of all lipids in plasma (roughly 300 gm. from the total plasma in seventy-two hours, equivalent to 900 calories per day). The levels of sugar in the blood fell to the upper limits of normal. The levels of ketones in the blood rose much less in this patient than has been observed in any other person on a comparable fast (maximal level 8.4 mg. per 100 ml.).

hour sample of blood was not obtained. Interpretation of the data includes the following: (1) The level of glycerides fell from 475 to 375 mg. per 100 ml. during the period from two to five hours, a rate in excess of 30 mg. per cent per hour. This rate was somewhat less than that for the patient A. Fon. (Fig. 3). (2) As in the case of A. Fon., the initial turnover rate for G. Har. was much slower than that for the nonhyperglyceridemic patients. (3) In contrast to patient A. Fon., the specific activity of the levels of glycerides in plasma continued to fall throughout the period of observation.

Patient M. Per., a forty-nine year old postmenopausal woman, had a skin eruption in 1953 which disappeared and then recurred. Constant glycosuria and creamy plasma were noted shortly afterward.

Our first examination of this patient revealed extensive xanthomas involving many portions of her body, of the eruptive, tuberosum and tendonosum varieties. In 1957, she experienced an episode of severe abdominal pain compatible in many of its manifestations with the diagnosis of acute pancreatitis. At this time the total content of lipids in plasma were in excess of 22,000 mg. per 100 ml. In the intervening years her response to a number of different diets has been evaluated. During one program of total abstinence from food, extending over a period of four days, a progressive decrease in all the lipids was observed (Fig. 7). By the fourth day, the level of

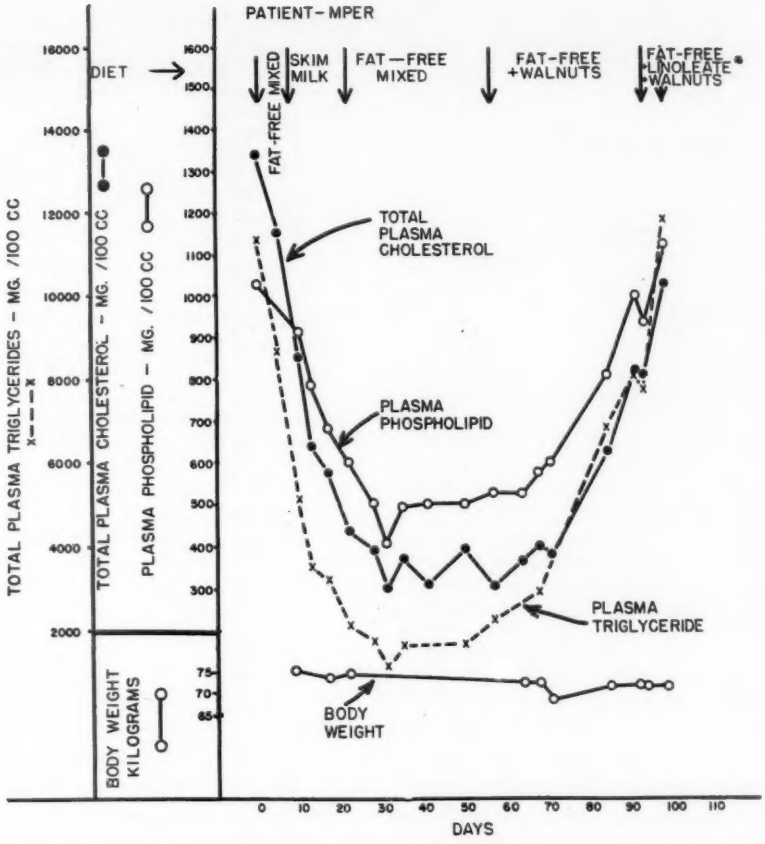

FIG. 8. With intake of any type of dietary fat, patient M. Per. experienced rapid increases in the levels of all lipids in plasma. On a fat-free diet, the levels of glycerides decreased toward (but not to) normal.

glucose in the blood was at the upper limits of normal. Under no other conditions was the level of sugar in the blood normal, and at no time was a tendency to develop ketoacidosis observed. Extreme resistance to insulin was manifested by this patient. The pattern of responses to mixed diets, with particular reference to a fat-free diet versus diets containing any type of fat, is shown in Figure 8.

Some of the data obtained following the administration of 10 μc. of C^{14}-tripalmitin in 50 ml. of cottonseed oil emulsion are provided in Figure 9. The following observations were made: (1) During the period from two to eight hours, the level of glycerides decreased from 2,675 to 2,325 mg. per 100 ml., a clearance rate of approximately 60 mg. per 100 ml. per hour. (2) The initial turnover rate was slow in comparison to that of the nonhyperglyceridemic patients, and the subsequent rate of removal was extremely prolonged.

Another hyperglyceridemic patient, E. Dix., first became aware of her disease in 1954 when she discovered yellow lesions on her left elbow. These were removed surgically and were found to be xanthomas. Subsequently, similar lesions appeared on other parts of her body. Plasma from this patient was milky in appearance.

The first examination of this patient, an obese woman fifty-five years of age, indicated that she was somewhat emotionally unstable.

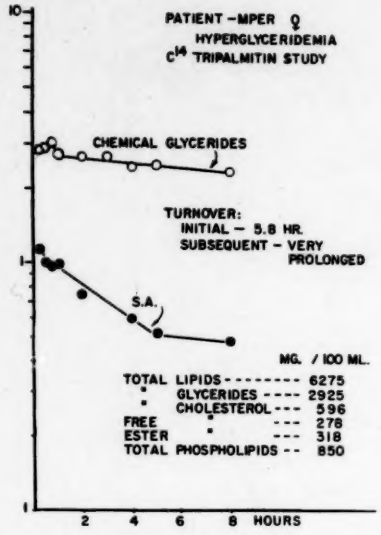

FIG. 9. The results of the C^{14}-tripalmitin study of patient N. Per. are shown. Under fasting conditions, following administration of C^{14}-tripalmitin, the initial and subsequent turnover of the labeled fat was much slower than normal. The levels of glycerides decreased at a rate of approximately 60 mg. per 100 ml. per hour. The initial clearance rate in our previous study (Fig. 7, Day 1) was slower than on subsequent days.

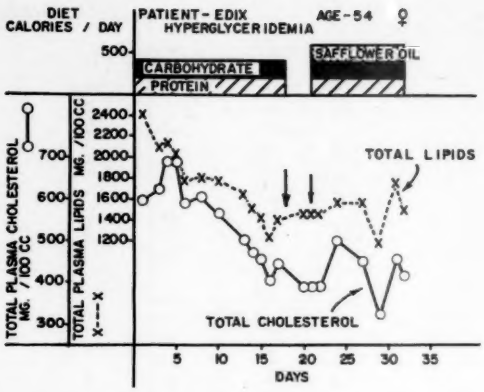

FIG. 10. During the administrations of a 400 calorie fat-free diet, patient E. Dix. experienced a slow decrease in the levels of all lipids in plasma over a two week period. Her plasma remained turbid and the levels of glycerides were elevated at all times. Addition of 22 gm. of safflower oil did not significantly increase the levels of lipids. The patient received no heparin or other pharmacologic agent during this study.

There was no evidence of hypothyroidism or nephrosis. The total content of lipids in plasma was, on occasion, in excess of 4,000 mg. per 100 ml. However, during the period when this patient was being fed a 400 calorie fat-free diet in the metabolic ward, the total content of lipids decreased from approximately 2,400 to 1,400 mg. per 100 ml. (Fig. 10). Evaluation of her responses to diet when she was not hospitalized was quite meaningless because of basic undependability of the patient in this regard. For a period of several weeks, subcutaneous injections of heparin were given daily; this treatment had no effect on the levels of lipids in plasma except for a marked elevation of the levels of free fatty acids.

At a time when her total lipids were 2,475 mg. per 100 ml., total glycerides, 544 mg. per 100 ml.; total cholesterol, 792 mg. per 100 ml. with a normal ester:free cholesterol ratio; and total phospholipids, 570 mg. per 100 ml., she was given 10 μc. of C^{14}-tripalmitin in 50 cc. cottonseed oil emulsion; the results are shown

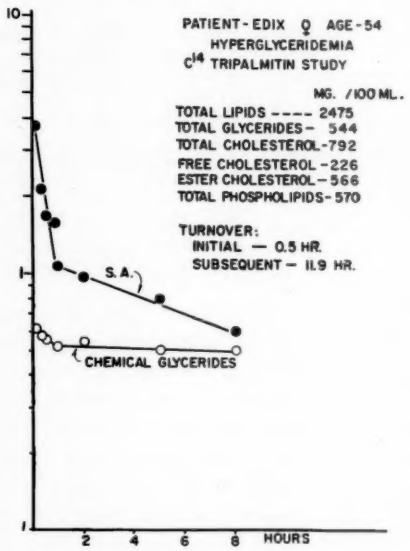

FIG. 11. Patient E. Dix, a hyperglyceridemic subject receiving heparin daily at the time the study was made, had a less abnormal initial turnover of labeled fat than any other patient with hyperglyceridemia; however, there was negligible decrease in the levels of glycerides in plasma during the fasting period of two to six hours. It is possible that the administration of heparin may have influenced the turnover time. This is being evaluated.

TABLE II
Data Pertaining to Tripalmitin Oxidation

Patient and Diagnosis	At Fifteen Minutes		Turnover of Glycerides (hr.)	Turnover of Fatty Acids (hr.)	$C^{14}O_2$ in First Twenty Minutes	
	Chemically Determined Glycerides (mg./100 ml.)	Specific Activity			Counts	Per Cent of Administered Dose
Nonhyperglyceridemia						
M. Mor. (epilepsy)	92	943	0.20	0.34	3,840	0.017
S. Gre. (hyperthyroidism)	74	2,230	0.12	0.90	9,900	0.045
Hyperglyceridemia						
G. Har.*	556	390	2.20	1.50	1,400†	0.013
A. Fon.	838	157	1.75	0.92	8,980	0.041
E. Dix.	575	206	0.50	2.20	12,440	0.056
M. Per.	2,825	113	5.80	1.00	0	0.0

* Total dose was 5 μc., given over a five minute period.
† $C^{14}O_2$ collected during period of zero to ten minutes; reported on a twenty minute basis.

in Figure 11. From these data, it appears that (1) the decrease in glycerides during the period from two to eight hours after injection was from 544 to 506 mg. per 100 ml., a rate of fall of approximately 6 mg. per hour, and (2) the initial turnover approached more closely that of the nonhyperglyceridemic subjects than any of the other hyperglyceridemic patients included in this study. Subsequent turnover, however, was significantly slower than that of either of the nonhyperglyceridemic patients and much slower than the patient with hyperthyroidism.

In Tables II and III are shown findings referable to oxidation of administered tripalmitin in the patients discussed herein. From the data in these tables, certain things seem apparent. (1) During the twenty minutes following initiation of administration of the C^{14}-tripalmitin-containing emulsion (50 cc. of emulsion was administered over a total period of five minutes to adults, and 25 cc. during the same period to the child) only a small percentage of the administered tripalmitin was oxidized in any one of the patients studied. (2) There appeared to be no consistent correlation between the rate of oxidation during this time and the total level of plasma glyceride, specific activity of glyceride, turnover of glyceride, or turnover of fatty acids resulting from the hydrolysis of glyceride. (3) In patient M. Per., with extremely elevated plasma glycerides and a very slow rate of turnover of glyceride, no measurable isotope was present in the expired air during the first twenty minutes. This patient also had the least amount of isotope in expired air during the first five hours of observation. (4) There is a striking contrast between the initial rate of oxidation of palmitic acid as compared to tripalmitin, both administered in the same amount and type of emulsion.

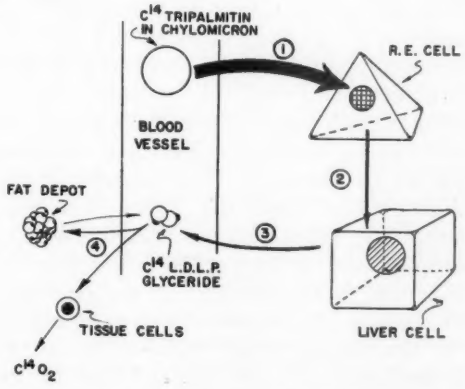

FIG. 12. The working hypothesis of metabolism of chylomicronous fat in the nonhyperglyceridemic subject is illustrated.

TABLE III
$C^{14}O_2$ Output During First Five Hours

Patient, Diagnosis and Agent	$C^{14}O_2$ Output (counts per minute)					
	0–20	45–60	105–120	165–180	224–240	285–300
Nonhyperglyceridemia						
M. Mor.						
Tripalmitin	192	5,875	7,480	....	....	....
Palmitic acid*	12,242	9,480	9,234	....	....	....
S. Gre.						
Tripalmitin	495	4,500	7,650	6,000	3,075	1,875
Hyperglyceridemia						
G. Har.						
Tripalmitin†	70	3,104	3,395	2,623	1,818	1,441
A. Fon.						
Tripalmitin	449	3,365	7,717	4,454	3,836	2,646
E. Dix.						
Tripalmitin	622	4,455	5,812	5,392	4,395	3,652
Palmitic acid*	6,889	10,268	8,665	....	....	....
M. Per.						
Tripalmitin	0	708	2,050	2,375	1,625	1,775

* Corrected to same dose as tripalmitin.
† Total dose 5 μc., given over a five minute period.

COMMENTS

If one were to assume that hyperglyceridemia in all persons is attributable to impaired removal of glyceride from the circulating plasma, he would anticipate that under comparable conditions with regard to fasting, intake of fat-free diets or intravenous infusion of emulsified isotopic tripalmitin, clearance of labeled or unlabeled glycerides would be decreased to approximately the same degree. This has not been the case in the patients studied. In an effort to obtain some explanation for the differences, let us consider first the data obtained from nonhyperglyceridemic patients.

In both of these subjects, there was a rapid removal of labeled glyceride, a secondary rise of the levels and then a more gradual decline (Figs. 1 and 2). Figure 12 is an attempt to explain diagrammatically the findings in these patients. One might postulate that the rapid removal of chylomicronous material is attributable largely to activity by reticuloendothelial (R.E.) cells, hepatic parenchymal cells or both and that the secondary rise is attributable to re-entry of the labeled glyceride which has been incorporated into specific lipoproteins by the hepatic cells. The labeled glyceride is gradually removed in hydrolyzed and nonhydrolyzed form by adipose tissue and by other tissue cells. Short or long term storage occurs in the former with subsequent oxidation by the latter. The fact that rapid removal of labeled chylomicronous material does not correspond with rapid oxidation (Table III) is compatible with this hypothesis. Further substantiation of this hypothesis obviously demands precise identification of the label in lipoprotein entities in plasma and in material obtained by biopsy of the liver. Such studies are planned.

If we accept this hypothesis and accept the general concept that the cottonseed oil emulsion follows somewhat the same rules as chylomicronous fat derived from the thoracic duct, we then have a basis for analysis of deviations from this pattern in the hyperglyceridemic patient.

Under the conditions of this study no fat entered the plasma from the digestive tract. If the level of glycerides in plasma is controlled by a "lipostat" mechanism, then on the basis of the observed values for the two nonhyperglyceridemic patients no fat should be mobilized from depots until the level in plasma

falls to 60 mg. per 100 ml. or below. If fat is mobilized from depots when the level in plasma is markedly in excess of this, some type of "lipostat disease" exists. (An analogy would be the excessive production of ACTH in patients with virilizing hyperplasia of the adrenal glands.)

It is apparent from the data given in Figures 3, 6, 9 and 11 that there was a continued decrease in the levels of glycerides in all the hyperglyceridemic patients except E. Dix. (Fig. 11) at rates varying from 30 to 60 mg. per 100 ml. per hour. The levels of lipids in plasma of E. Dix. (Fig. 10) did not become normal even when she was fed a fat-free calorically restricted diet for a period of two weeks while on the metabolic ward. During the labeled tripalmitin study of this patient, the labeled chylomicronous fat was cleared at rates approaching normal, and her initial rate of oxidation of the labeled fat was the highest of any of the four hyperglyceridemic patients (Table II). These findings suggest that this patient mobilizes glyceride steadily in the presence of a high level of glycerides in plasma, i.e., she has marked lipostat disease. They also suggest that chylomicronous glyceride is removed rapidly and oxidized readily. Abnormality exists in the mechanism for removing glyceride bound to other lipoprotein or lipoproteins.

Glycerides are cleared from plasma rapidly in patient M. Per. under conditions of almost no intake of fat (Fig. 8). This clearance occurs until a level of approximately 2,000 mg. per 100 ml. is reached, at which time lipostat disease becomes apparent. Data obtained from the labeled tripalmitin study of this patient (Fig. 9) together with the data in Figures 7 and 8 are compatible with the concept of impairment of metabolism of both chylomicron glyceride and "lipoprotein glyceride." The combination of isotopic and ultracentrifugal studies will clarify this matter.

In the data concerning patient A. Fon. (Fig. 3), there is little to suggest lipostat disease. The findings could be compatible with the concept that chylomicronous glyceride and glycerides, derived directly from chylomicrons (by way of initial hepatic metabolism?),

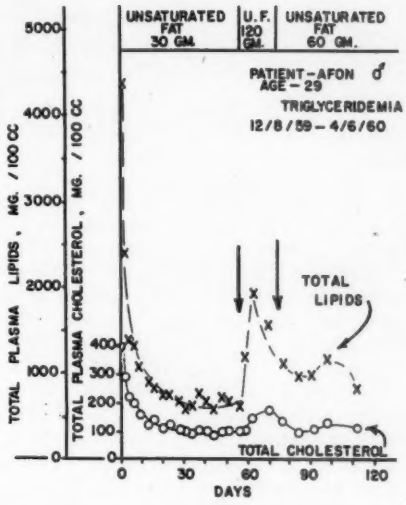

FIG. 13. Analyses of plasma from patient A. Fon., while taking a diet containing 30 gm. of predominantly polyunsaturated fat, indicated that the levels of all lipids fell to normal and turbidity disappeared.

may not be metabolized as well as glyceride entering the plasma from adipose tissue. The data given in Figure 13 are in accordance with this interpretation.

The data obtained from patient G. Har. (Fig. 6) also suggest that no lipostat disease exists, that the basic defect is associated with the handling of chylomicronous fat and that the handling of nonchylomicronous lipoprotein glyceride is normal or nearly normal.

Portions of these interpretations are speculative. The purpose of this report is only to indicate that under the general heading of hyperglyceridemia more than one syndrome exists. Some of these appear to be associated with primary difficulty in metabolizing chylomicronous glyceride, others with difficulties relating to metabolism of one or more of the glyceride-carrying lipoproteins of greater density than chylomicron. Lipostat disease appears to be a significant part of some of these syndromes. Whether or not such a disease is associated with hormonal deficiency or excess, or with disturbance in other aspects of the normal check and balance system for regulation of levels of glyceride in plasma, will be determined by further study. It is probable

that progressively better understanding of the gross abnormalities associated with the hyperglyceridemias will contribute to better understanding of lesser degrees of hyperlipidemia associated with atherosclerosis and other diseases.

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Fatty Acid Distributions in Serum Lipids and Serum Lipoproteins

FRANK T. LINDGREN, PH.D.,* ALEX V. NICHOLS PH.D.† AND ROBERT D. WILLS‡

WITH THE exceptions of unesterified cholesteryl and unesterified fatty acids the serum lipids consist almost entirely of esters of long chain fatty acids. These lipids, such as cholesterol esters, glycerides and phospholipides, are biochemically complex mixtures. Thus, for some of these classes of lipids there are at least as many chemically distinct compounds as there are specific fatty acids present within each class. Since these serum lipids may be quantitatively and qualitatively abnormal, as occurs in certain of the hyperlipemic and hyperlipoproteinemic states, it is of potential importance that the fatty acid distribution within each of the major serum lipids be studied in detail. Furthermore, since nearly all the serum lipids are present as part of the structure of several groups of lipoproteins, some of which are specifically elevated in certain disease states,[1] it is of additional interest to determine what differences, if any, exist within the same lipid class taken from each of these different serum lipoprotein groups.

Until recently fatty acid analysis of serum lipids, either by the older chemical technics or by the more recent gas-liquid chromatographic technics, has required sample sizes the order of milligrams. However, after fractionating samples of human serum into chemical and lipoprotein fractions these relatively large milligram size samples are not readily available. Recent progress in gas-liquid chromatography has allowed analysis of very small lipid samples primarily as a result of improvement in detection devices. The use of the beta particle detector with argon as a carrier gas[2] now permits analysis of the order of $^1/_{100}$ the sample size required of the thermal conductivity detection systems. Also, improvements in the chromatographic column itself have further facilitated fatty acid analysis. Thus, using certain polyesters of diethylene glycol[3] as a liquid phase, the time required for complete analysis of fatty acids has been reduced severalfold.

Previous investigators[4,5] have reported on the fatty acid composition of phospholipid, unesterified fatty acid and a fraction containing glyceride plus cholesteryl esters. Our present study is an effort to provide a more complete fatty acid analysis of each fatty acid-containing chemical fraction isolated both from whole serum and from each of the major serum lipoprotein fractions.

METHODS

Samples of venous blood for both serum and lipoproteins were obtained from nonfasting normal adults employed at the Livermore and Berkeley sites of the Lawrence Radiation Laboratory. Prior to the drawing of blood, each subject maintained his normal dietary pattern.

Ultracentrifugal Fractionation of Serum

In the native serum, lipids exist entirely in the form of lipoproteins. Although these

From the Donner Laboratory of Medical Physics, Division of Medical Physics, Department of Physics and the Lawrence Radiation Laboratory, University of California, Berkeley, California.

* Research Associate in Biophysics, Donner Laboratory of Medical Physics, Department of Physics, University of California, Berkeley, California; † Research Biophysicist and Lecturer in Medical Physics and Biophysics, Donner Laboratory of Medical Physics, Department of Physics, University of California, Berkeley, California; ‡ Laboratory Research Technician, Donner Laboratory of Medical Physics, Department of Physics, University of California, Berkeley, California.

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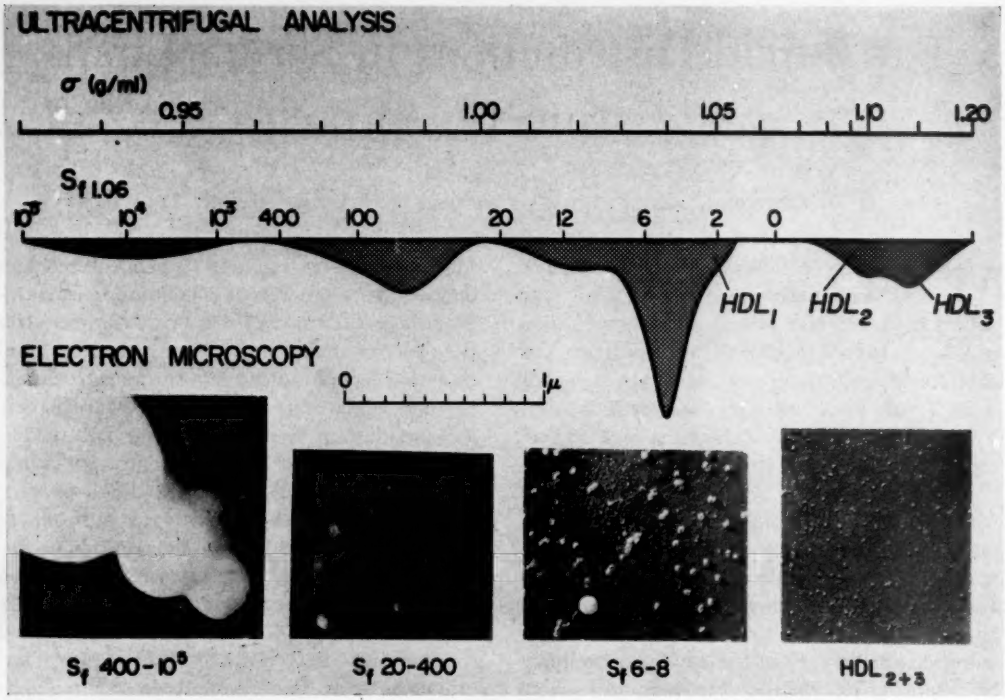

FIG. 1. Schematic diagram of the total serum lipoprotein spectrum. Electron micrographs illustrate the physical sizes of each broad lipoprotein group. The corresponding hydrated densities and ultracentrifugal flotation rates (for the low density class only) are shown for each region of the lipoprotein spectra. From: FREEMAN, N. K., LINDGREN, F. T. and NICHOLS, A. V. Progress in the Chemistry of Lipids and Other Substances, vol. 6. New York, 1961. Pergamon Press, Inc.[19]

lipoproteins are complex macromolecules which vary in size, shape and chemical composition, they fall into three or four broad categories. Figure 1 illustrates the distribution of sizes (as determined by electron microscopy) and the corresponding distribution in hydrated density for each region of the serum lipoprotein spectrum (expressed ultracentrifugally). For the present study, the serum was fractionated into three major lipoprotein groups.

Separation of the serum into lipoprotein fractions was achieved by a method[6] of successive preparative ultracentrifugation (at densities,* before ultracentrifugation, of 1.006, 1.070 and 1.218 gm./ml.). However, in the present study, sodium bromide (NaBr) was used instead of deuterium oxide (D_2O), sodium chloride (NaCl) and sodium nitrate ($NaNO_3$) to provide the appropriate density increments. The procedure, carried out at 15° to 18°c., utilizing a Spinco Model L ultracentrifuge and a 40.3 rotor is as follows: initially 6 ml. aliquots of serum are ultracentrifuged for twenty-four hours at 40,000 r.p.m. (108,000 g) yielding a top fraction containing lipoproteins which are less dense than 1.006 gm./ml. This s_f° $20-10^5$ lipoprotein fraction is quantitatively removed in 1 ml. and a second 1 ml. fraction is also removed. The remaining 4 ml. bottom fraction is mixed with 2 ml. of a 0.195 molal solution of NaCl, 2.69 molal NaBr solution of density 1.182 gm./ml. giving a resultant small molecule solution of density 1.070 gm./ml. A second twenty-four hour ultracentrifugation of this mixture results in a top 1 ml. fraction of density 1.062 gm./ml. containing the s_f° $0-20$ lipoprotein class. This lipo-

* All densities are referred to 20°c.

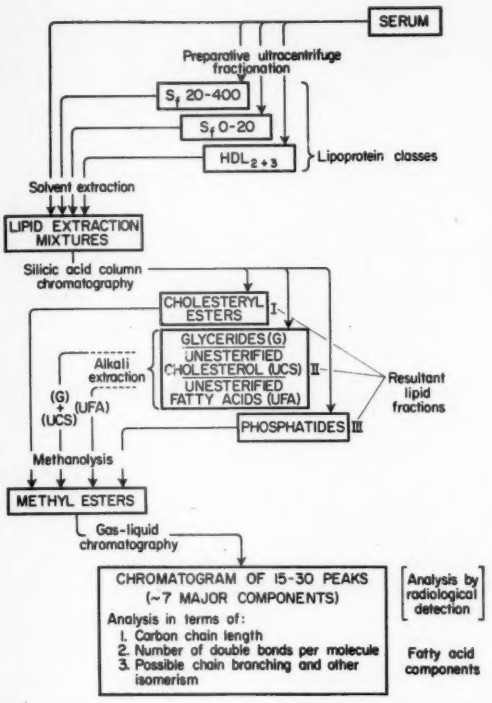

FIG. 2. The lipid analytic procedure used in the fatty acid composition studies of both the serum and the serum lipoprotein fraction. From: FREEMAN, N. K., LINDGREN, F. T. and NICHOLS, A. V. Progress in the Chemistry of Lipids and Other Substances, vol. 6. New York, 1961. Pergamon Press, Inc.[19]

protein fraction is quantitatively removed in 1 ml. and a second reference 1 ml. fraction again is removed. The remaining 4 ml. bottom fraction is mixed with 2 ml. of a 0.195 molal solution of NaCl, 7.60 molal NaBr solution of density 1.483 gm./ml. yielding a density of 1.218 gm./ml. A final twenty-four hour ultracentrifugation yields a top 1 ml. fraction of density 1.203 gm./ml. containing the high density 2 and 3 class lipoproteins. These HDL–2 and 3 lipoproteins are removed in the top 1 ml. and a second 1 ml. fraction is taken for reference. The remaining bottom fraction is the ultracentrifugal residue. The differences observed between the densities of initial solutions and the final top 1 ml. fractions after each run are the result of the sedimentation of the salts during each centrifugal process.

TABLE I
Results of Calibration with a Standard Methyl Ester Mixture

Methyl Ester Component	Sample No. 1	2	3	4	Actual Weight (%)
pre 16:0	0.1	0.1	0.1	0.1	...
16:0	25.2	25.5	24.8	24.8	24.7
18:0	16.3	16.0	16.0	15.4	16.7
18:1	20.9	21.0	21.1	21.6	20.5
18:2	17.6	17.6	18.0	18.0	17.3
18:3	3.8	3.7	3.7	3.8	4.7
18:3–20:4	0.6	0.6	0.6	0.6	...
20:4	15.5	15.6	15.8	15.7	16.2
Total mass analyzed (μg.)	87.6	64.9	43.6	23.3	...

Lipid Analytic Procedure

The analytic scheme for both serum and serum lipoprotein fractions is shown diagrammatically in Figure 2. Lipids were extracted from the serum sample and lipoprotein samples by a modification of a method of Sperry.[7] Separation by silicic acid chromatography of the lipid samples into three fractions containing cholesteryl esters, glycerides plus cholesterol plus unesterified fatty acids and phospholipids was carried out by a procedure described by Freeman.[8] Isolation of unesterified fatty acids from the second fraction was accomplished by the alkali extraction method of Borgstrom.[9] Following these separations, each lipid fraction was methylated by the transesterification method of Stoffel.[10] Gas-liquid chromatography of the fatty acid methyl esters was performed on an apparatus described by Upham.[11] This apparatus employs a 52 inch glass column (6 mm., inside diameter) packed with 48 to 65 mesh Chromosorb in which is incorporated LAC-2R-728,* 30 per cent by weight. Prior to use each column is pre-bled at 195°c. for approximately three to four weeks in another thermal chamber.

Gas-Liquid Chromatographic Analysis

Calibration of our gas chromatographic apparatus was made using both Hormel gas

* Obtained from Cambridge Industries Company, Inc.

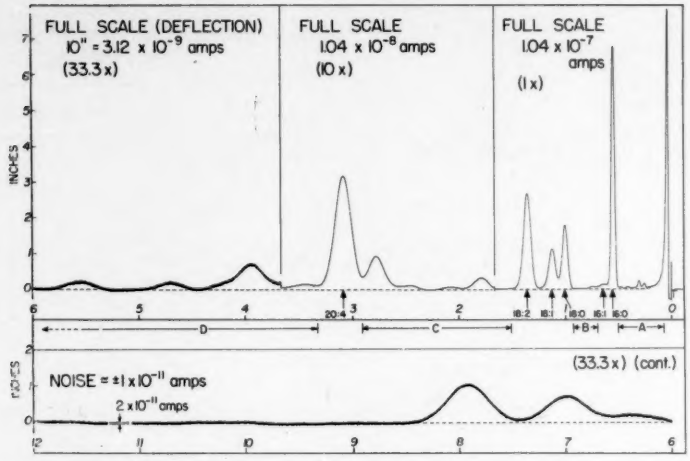

FIG. 3. Representative chromatogram obtained from a total serum phospholipid sample (Case 12). Usable dynamic range approximately 10^4 to 1 (maximum usable signal = 1.04 x 10^{-7} amps and noise approximately 1 x 10^{-11} amps). From: FREEMAN, N. K., LINDGREN, F. T. and NICHOLS, A. V. Progress in the Chemistry of Lipids and Other Substances, vol. 6. New York, 1961. Pergamon Press, Inc.[19]

chromatographic methyl ester mixtures and mixtures prepared from individual methyl ester samples obtained from the Hormel Institute. Table I shows the results of such a calibration for a mixture of methyl esters of from 23 to 88 μg. in size. Initially, in determining fatty acid components from each gas chromatogram we calculated areas under each component by both triangulation and planimetry and made small corrections on the basis of peak height and elution times. However, our present procedure using punched card technics has demonstrated equivalent results with the added advantage of facility of data manipulation and calculation. The data presented have been processed by our punched card technic.[12] This technic evaluates the mass of each chromatographic component by the product of its peak height and elution time. An additional calculation to correct for small deviations from linearity over all ranges of detection currents is also made.

In reporting our data we have adopted the classification proposed by Dole[4] in which fatty acid identification is given by carbon chain lengths followed by the number of double bonds per molecule, if any. The elution time is reported relative to that of methyl stearate. Since the number of resolvable components present in each gas chromatogram number at least fifteen to thirty there is clearly a formidable problem of tabulating, presenting and comparing data. Therefore, to allow comparison of our data certain simplifications have been made by grouping together several components or classes of components. We have four such classes of unidentified components. However, 80 to 90 per cent by calculated weight of the component fatty acids present on each chromatogram may be directly identified with known methyl ester elution times. The remaining 10 to 20 per cent have been grouped into four classification bands as follows: pre 16:0 (A), 16:1–18:0 (B), 18:2 to 20:4 (C) and post 20:4 (D).

Figure 3 shows a representative chromatogram of fatty acid methyl ester obtained from a serum phospholipid sample. Since diffusion of the methyl ester component results in pronounced broadening of the late components (as a first approximation the peak width is proportional to the elution time) it is desirable to simultaneously analyze a sample at two levels of sensitivity using either

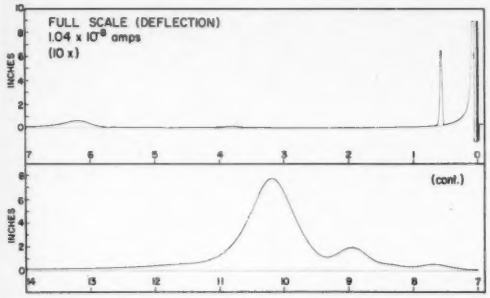

FIG. 4. Chromatogram of 75 μg. unesterified cholesterol and 2.09 μg. methyl palmitate. Sample was injected in 7.5λ benzine. Mass response for unesterified cholesterol in terms of area is approximately equivalent to that of methyl palmitate.

two separate recorders or a dual channel recorder. We have found a factor of ten between recorders to be satisfactory for most routine analysis. However, to analyze adequately components beyond methyl arachidonate additional sensitivity is frequently required.

The presence of unesterified cholesterol in the methyl ester samples from both the cholesteryl ester and glyceride-containing lipid fractions definitely interferes in the gas chromatographic analysis. Figure 4 shows the chromatographic pattern of unesterified cholesterol. When present, cholesterol interferes beyond stearate times of approximately six as well as preventing any subsequent analysis before twelve stearate times have passed. However no interference is encountered before six stearate times. Our data presented here are for methyl ester samples without unesterified cholesterol removal and therefore represent adequate analysis of these fractions only up to six stearate times. Both silicic acid chromatography and microsublimation[10] allow separation of the methyl esters from unesterified cholesterol. However, each of these procedures may introduce both losses and fractionating effects unless scrupulous control measures are maintained.

It is understood that fatty acid composition studies involving only one gas chromatographic analysis is limited in scope. Ideally, a more rigorous and complete identification of each component should be made, yet comparison of fatty acid distributions made by only one procedure are valid. Table II lists the possible fatty acids that may be included in each of the four unidentified groups. These data[13,14] are for similar liquid phases and the relative stearate times are given for temperatures a few degrees higher than our operating conditions (193°C.).

RESULTS OF SERUM FRACTIONS

The results for the lipid fractions obtained from seven serum samples are given in Tables IIIA, B and C and IV. All values are given as weight per cent of total methyl esters determined from each gas chromatogram. The results show a definite pattern of fatty acid composition for each chemical fraction obtained from all seven subjects. Some variations in fatty acid composition for the same chemical fraction is observed from person to person. However, a definite pattern of composition is observed among all subjects for each chemical class. For the cholesteryl ester fractions the predominant fatty acids are linoleic (55 per cent), oleic (18 per cent), palmitic (10 per cent) and arachidonic (7 per cent). Thus, cholesteryl esters are an important biochemical form for the transport of the more highly unsaturated fatty acids. In contrast, serum glycerides contain a considerably higher content of the more saturated fatty acids. The principle fatty acids present in the glyceride containing fractions are oleic (39 per cent), palmitic (30 per cent) and linoleic (16 per cent). The phospholipid fractions show another distinct fatty acid pattern. The major fatty acids are palmitic (33 per cent), linoleic (22 per cent), stearic (14 per cent), oleic (12 per cent) and arachidonic (9 per cent). Compared to the other chemical fractions of serum, phospholipids are associated with an unusually high content of both palmitic and stearic acid. Finally, the unesterified fatty acid fractions primarily transported in the serum bound with serum albumin show yet another fatty acid distribution. The major fatty acids for this fraction are oleic (26 per cent), palmitic (25 per cent) and linoleic (16 per cent).

The aforementioned results for each chemi-

TABLE II

Comparison of Retention Volume Data of Identified Fatty Acid Methyl Esters (relative to methyl stearate) with Typical Retention Volumes as Obtained from Chromatogram Reproduced in Figure 3

Methyl Ester Component	Results of Farquhar et al.[13]: Reoplex 400 (197°c.) (Menhaden Oil)	Results of Farquhar et al.[13]: PDEGA* (197°c.) (Menhaden Oil)	Results of Hawke, Hansen and Shorland[14] PDEGS† (200°c.)	Our Data: PDEGS (193°c.) Serum No. 12 (Phospholipid)	Classification Bands
8:0	...	...	0.055	...	
9:0	...	...	0.078	...	
10:0	...	...	0.104	...	
10:1	...	...	0.133	...	
11:0	...	...	0.136	...	
12:0	0.180	0.165	0.184	0.175	A
13:0	...	0.222	0.236	0.254	
14:0	0.320	0.302, 0.252$^{\alpha}$‡	0.324	0.302	
14:1	0.360	0.343	...	0.352	
15:0	0.430	0.401, 0.367$^{\beta}$	0.424	0.405	
	...	...	...	0.475	
16:0	0.570	0.550, 0.472$^{\alpha}$, 0.402$^{\gamma}$	0.573	0.544	
16:1	0.640	0.634	...	0.630	
17:0	0.740	0.733, 0.676$^{\beta}$	0.741	0.736	
16:2 (Δ6,9)	0.788	0.785	...	...	
16:2 (Δ9,12)	0.788	0.785	...	0.831	B
16:3 (Δ6,9,12)	0.900	0.904	...	...	
16:3 (Δ7,10,13)	0.900	0.904	...	...	
18:0	1.00	1.00	1.000	1.000	
18:1 (Δ9)	1.11	1.12	1.120 (trans), 1.146 (cis)	1.118	
18:2 (Δ9,12)	1.32	1.34	1.404	1.349	
18:2 (Δ6,9)	1.40	1.43	...	...	
19:0	1.32	1.35, 1.24$^{\beta}$	1.301	...	
18:3 (Δ9,12,15)	1.66	1.72	1.814	1.548	
18:3 (Δ6,9,12)	1.66	1.72	...	1.765	
20:0	1.78	1.82, 1.59$^{\alpha}$, 1.35$^{\alpha}$	1.780	...	
18:4 (Δ6,9,12,15)	1.91	1.97	...	...	
20:1 (Δ11)	1.96	2.02	...	2.065	C
20:2 (Δ8,11)	2.24	2.32	...	...	
20:2 (Δ11,14)	...	2.45	...	2.432	
21:0	...	2.46, 2.29$^{\beta}$	2.362	...	
20:3 (Δ8,11,14)	2.68	2.76	...	2.761	
20:4 (Δ5,8,11,14)	2.90	3.04	3.117	3.073	
22:0	...	3.27, 2.91$^{\alpha}$	3.181	...	
22:1 (Δ13)	...	...	3.641	3.464	
20:5 (Δ5,8,11,14,17)	3.67	3.85	...	3.927	
23:0	...	...	4.44	...	
22:4	5.01	...	...	4.75	D
24:0	...	...	5.67	5.55	
22:5	5.80, 6.30	...	...	6.20	
22:5 (Δ7,10,13,16,19)	6.67	7.00	...	6.50	
22:6 (Δ4,7,10,13,16,19)	7.40	7.75	...	7.01	
26:0	...	...	10.30	7.90	

NOTE: Position of double bonds (as assigned by Farquhar et al. and Hawke, Hansen and Shorland) are numbered from the carboxyl carbon (number 1). Thus, oleic acid 18:1 (Δ9) has its double bond between carbon atoms 9 and 10.

* PDEGA = polydiethylene glycol adipate. † PDEGS = polydiethylene glycol succinate. ‡ Identification of branched chain isomerism (Ahrens et al.) by superscript is as follows: α = iso(terminal isopropyl group); β = ante-iso(terminal isobutyl group); γ = neo(terminal neopentyl group).

TABLE III
Fatty Acid Composition

Methyl Ester Component	Case No. 1	2	3	4	5	6	7	Mean
A. Serum Cholesteryl Esters								
pre 16:0 (A)*	4.9	2.4	3.2	1.9	2.0	1.3	5.5	3.0
16:0	13.3	10.3	8.6	9.9	8.5	8.6	10.6	10.0
16:1	7.4	2.4	2.3	3.0	1.7	2.7	3.0	3.2
16:1–18:0 (B)	2.0	0.7	1.3	0.7	0.7	0.6	1.8	1.1
18:0	1.6	1.1	1.2	1.0	1.0	0.8	1.9	1.2
18:1	24.3	18.2	15.6	17.8	13.4	18.0	17.3	17.8
18:2	35.1	58.3	60.1	53.0	64.8	62.1	53.4	55.3
18:2–20:4 (C)	3.4	1.1	2.4	3.2	1.6	1.4	2.1	2.1
20:4	7.1	5.2	4.8	8.2	5.9	3.9	4.3	5.6
post 20:4 (D)	1.0	0.4	0.6	1.2	0.5	0.5	...	0.6
Total mass analyzed (μg.)	74.4	81.6	116.8	105.5	146.1	166.0	119.9	...
B. Serum Glyceride-Containing Fraction								
pre 16:0 (A)	3.7	3.5	4.1	4.4	2.8	1.9	3.8	3.5
16:0	35.2	27.1	28.9	31.9	26.0	33.1	26.5	29.8
16:1	5.3	3.8	3.3	4.0	3.3	3.2	3.3	3.7
16:1–18:0 (B)	1.4	1.3	1.1	1.3	1.0	0.7	0.9	1.1
18:0	4.5	5.2	4.1	5.2	4.9	3.3	5.1	4.6
18:1	38.8	39.6	37.5	36.9	37.9	41.0	42.0	39.1
18:2	9.3	17.4	19.3	13.0	20.9	14.7	15.7	15.7
18:2–20:4 (C)	1.1	1.0	0.8	1.4	1.4	1.1	1.5	1.2
20:4	0.8	1.3	1.0	1.9	1.7	1.0	1.3	1.3
post 20:4 (D)	...	...	...	...	...	...	...	...
Total mass analyzed (μg.)	65.0	59.0	53.5	60.4	58.8	76.1	56.2	...
C. Serum Phospholipid Fraction								
pre 16:0 (A)	1.9	1.7	1.3	2.4	2.3	1.6	2.4	2.0
16:0	30.2	33.3	38.2	34.1	33.8	31.2	31.7	33.2
16:1	1.4	0.9	0.9	1.1	1.1	1.0	1.2	1.1
16:1–18:0 (B)	1.0	0.9	0.9	0.8	1.1	0.9	1.0	0.9
18:0	15.3	15.2	12.8	14.2	15.1	12.6	14.7	14.3
18:1	17.0	10.3	10.5	9.7	9.9	12.2	13.5	11.9
18:2	20.6	24.1	23.8	18.4	21.8	22.3	22.1	21.9
18:2–20:4 (C)	3.4	2.7	2.7	4.6	3.9	2.2	3.6	3.3
20:4	9.4	9.0	7.7	13.0	10.6	7.4	8.3	9.3
post 20:4 (D)	...	1.9	1.2	1.6	0.5	7.6	1.4	2.0
Total mass analyzed (μg.)	24.0	75.6	70.9	68.9	64.3	54.4	71.3	...

* Letters between parentheses indicate classification bands.

cal fraction show very similar fatty acid distributions for all subjects studied. These data would suggest that the general dietary patterns (home diet) of these subjects were similar with regard to the composition of dietary fat. The data of Ahrens et al.[15] reveal significant differences in fatty acid distribution during long-term dietary periods in which different kinds of fats were fed. Thus, if significant differences in dietary fats were in-

TABLE IV
Fatty Acid Composition of the Serum Unesterified Fatty Acid Fraction

Methyl Ester Component	Case No.				Mean
	8	9	1	4	
pre 16:0 (A)*	8.7	8.4	6.6	6.6	7.6
16:0	21.6	25.6	24.0	28.7	25.0
16:1	3.5	5.0	4.7	4.0	4.3
16:1–18.0 (B)	2.0	3.4	1.6	1.8	2.2
18:0	9.8	10.6	9.7	10.0	10.0
18:1	24.4	23.6	29.7	26.3	26.0
18:2	21.5	13.8	12.9	15.4	15.9
18:2–20:4 (C)	5.3	4.5	4.1	2.4	4.1
20:4	0.6	1.7	2.8	2.9	2.0
post 20:4 (D)	2.7	3.5	6.8	2.0	3.7
Total mass analyzed (μg.)	41.0	22.0	63.4	41.3	...

* Letters between parentheses indicate classification bands.

volved one would expect to note differences in fatty acid composition from person to person. Furthermore, one would not expect to observe any significant influence on fatty acid distribution from the fat content of the previous meal even though it may have departed from the general pattern of fatty acid content. This would be inferred from the work of Dole and associates[4] who observed that the serum fatty acid composition was relatively stable to acute fat loads of different fatty acid composition.

FATTY ACIDS IN LIPIDS OF THE MAJOR LIPOPROTEIN CLASSES

Results of this preliminary study were obtained from serum fractionated from three normal adults (Cases 11 through 14). Cases 11 and 13 represent serum samples obtained from the same person one month apart. Marked differences in fatty acid composition were observed between these two samples.

Table vA shows the fatty acid distribution obtained from the cholesteryl ester moiety of the three principal lipoprotein classes. The principal fatty acids are linoleic, oleic and palmitic. Comparison of the fatty acid distributions among the different lipoproteins for each subject revealed close similarity between the S_f° 0−20 and HDL-2 and 3 class lipoproteins. However, for the four cases studied, there appears to be a different pattern for the very low density s_f° 20–10^5 lipoprotein group. These differences may be characterized by an elevated level of palmitic and oleic acid and a reduced level of linoleic acid relative to the two higher density lipoprotein classes. Also, although of relatively low abundance, stearic acid (see Case 12) is elevated and arachidonic acid is lowered within the s_f° 20–10^5 class. When the four samples are compared with one another, the data show general similarity in fatty acid distribution for both the s_f° 0–20 and HDL-2 and 3 groups. The greatest variability exists within the principal fatty acid components of the s_f° 20–10^5 lipoprotein group.

Table vB shows the fatty acid distribution observed for the glyceride containing lipid fraction obtained from each lipoprotein group. From sample to sample, these fractions revealed comparatively minor differences in fatty acid distribution. If we consider each individual sample, we observe very close similarity between the s_f° 0−20 and the HDL-2 and 3 lipoprotein classes. In contrast, there may be some differences in the glyceride fraction of the s_f° 20–10^5 lipoproteins. This fraction shows a somewhat higher content of linoleic acid (with the exception of Case 11). In all lipoprotein fractions, the prepalmitic acid group are the highest observed for any of the three chemical fractions isolated from any of the other lipoprotein groups. However, there are uncertainties in the measurement of the short chain fatty acids since they may be selectively lost during the processing of the lipid samples because of their higher volatility. The use of a solvent to inject the methyl ester samples introduces baseline uncertainties in the very early portion of the chromatogram and usually does not allow measurement of components of shorter chain length than 10:0.

Table vC reveals the fatty acid distribution present in the phospholipid fractions obtained from each lipoprotein class. With the exception of Case 12 the fatty acid distribution of the phospholipid moiety of the three ultracentrifugal fractions of each sera are similar. However, Case 12 does reveal similar fatty

TABLE V

Fatty Acid Composition from Each Lipoprotein Class

Methyl Ester Component	S_f° 20−10^5				Mean	S_f° 0−20				Mean	HDL-2 and 3				Mean
	Case No.					Case No.					Case No.				
	11	12	13	14		11	12	13	14		11	12	13	14	
A. Cholesteryl Ester Fraction															
pre 16:0 (A)*	0.3	5.5	3.4	1.7	2.7	6.7	2.3	1.5	1.1	2.9	3.8	2.7	1.8	1.4	2.4
16:0	13.8	23.6	28.1	25.5	22.8	10.7	10.8	10.8	10.9	10.8	10.8	10.6	10.1	11.2	10.7
16:1	4.5	2.2	1.9	2.9	2.9	4.1	3.1	2.7	3.1	3.3	3.7	2.8	2.9	3.3	3.2
16:1–18:0 (B)	...	1.9	1.2	0.9	1.0	1.3	1.2	0.7	0.8	0.9	0.7	0.9	0.8	0.6	0.8
18:0	2.4	10.9	6.8	4.2	6.1	1.7	1.2	1.2	1.3	1.3	1.2	1.1	1.1	1.4	1.2
18:1	27.2	28.0	36.7	46.3	34.6	17.4	19.6	19.8	20.6	19.3	17.2	18.6	18.7	21.4	18.9
18:2	46.5	23.9	18.5	15.3	26.0	49.7	53.0	54.7	50.0	51.9	52.4	53.4	55.6	47.5	52.2
18:2–20:4 (C)	2.5	2.0	1.7	1.9	2.0	3.5	3.9	2.0	4.0	3.3	3.4	2.5	2.2	3.7	2.9
20:4	3.0	2.0	1.4	1.3	1.9	4.2	5.6	6.0	7.2	5.8	5.7	6.0	6.2	7.5	6.3
post 20:4 (D)	...	...	0.3	...	...	0.7	0.7	0.6	1.0	0.8	1.2	1.5	0.7	1.9	1.3
Total mass analyzed (μg.)	39.9	58.6	76.4	87.7	...	68.8	44.4	52.8	48.1	...	45.1	56.4	51.2	58.0	...
B. Glyceride-Containing Fraction															
pre 16:0 (A)	3.2	7.1	4.1	3.4	4.5	7.4	7.2	5.8	5.2	6.4	4.9	8.0	4.7	4.3	5.5
16:0	28.2	24.2	24.8	21.7	24.7	24.6	25.0	26.8	23.3	24.9	28.9	22.9	24.2	22.0	24.5
16:1	3.1	3.6	3.8	5.8	4.1	4.2	4.2	3.5	5.2	4.2	3.9	3.6	3.5	4.9	4.0
16:1–18:0 (B)	1.1	1.1	1.0	1.0	1.1	2.1	1.6	1.4	1.6	1.7	2.0	2.1	1.3	1.1	1.6
18:0	6.7	3.9	2.5	2.4	3.9	5.4	5.7	3.8	4.2	4.8	5.9	5.4	4.4	2.9	4.6
18:1	40.0	30.4	31.7	38.7	35.2	36.3	29.7	36.1	40.4	35.6	39.1	28.7	35.8	39.9	35.9
18:2	16.2	23.9	27.7	22.1	22.5	16.2	19.7	18.3	14.3	17.2	13.3	18.2	20.7	16.4	17.1
18:2–20:4 (C)	1.2	3.6	2.8	3.5	2.8	2.8	3.2	2.2	3.7	3.0	2.1	5.6	2.7	5.0	3.8
20:4	0.4	1.5	1.0	1.4	1.1	1.0	2.0	1.3	1.4	1.4	...	2.1	1.3	1.9	1.3
post 20:4 (D)	...	0.8	0.5	...	0.3	...	1.7	1.0	0.6	0.8	...	3.6	1.6	1.6	1.7
Total mass analyzed (μg.)	57.4	57.1	64.3	54.3	...	40.0	58.4	69.9	43.6	...	32.5	43.8	47.8	69.1	...
C. Phospholipid Moiety															
Fatty Acid Component															
pre 16:0 (A)	1.8	2.4	2.1	2.6	2.3	2.7	3.4	1.9	2.6	2.6	4.2	2.9	3.4	2.0	3.1
16:0	36.3	39.9	25.9	43.7	36.4	32.6	28.2	27.5	40.2	32.1	28.6	25.0	24.6	42.6	30.2
16:1	1.3	1.5	1.3	1.1	1.3	2.6	1.3	1.1	1.4	1.6	1.3	1.3	1.5	1.7	1.4
16:1–18.0 (B)	1.4	2.0	1.5	1.8	1.7	1.4	1.3	1.1	1.4	1.3	1.2	1.7	1.8	1.6	1.6
18:0	16.5	23.6	14.7	20.2	18.7	13.2	14.4	13.4	22.7	15.9	15.1	14.0	11.9	23.4	16.1
18:1	11.2	17.3	10.8	13.4	13.2	9.6	10.8	10.6	16.8	12.0	11.2	10.5	10.6	17.4	12.4
18:2	20.6	7.9	26.0	10.0	16.1	19.6	27.2	25.0	8.1	20.0	23.8	28.2	22.8	8.2	20.8
18:2–20:4 (C)	4.8	2.8	4.0	2.4	3.5	5.0	3.7	4.5	2.3	3.9	4.4	3.9	5.4	2.5	4.1
20:4	6.1	1.1	9.0	0.8	4.3	7.0	8.9	9.7	1.1	6.7	10.3	11.4	11.7	0.7	8.5
post 20:4 (D)	...	1.7	4.6	4.0	2.6	6.5	0.9	5.4	3.4	4.0	...	1.2	6.4	...	1.9
Total mass analyzed (μg.)	74.8	37.5	50.8	43.4	...	111.4	37.1	43.6	34.2	...	49.2	30.4	51.7	37.9	...

* Letters between parentheses indicate classification bands.

acid composition for the S_f° 0−20 and HDL-2 and 3 lipoprotein fractions. The S_f°20−10^5 lipoproteins in Case 12 show a higher palmitic and stearic acid content as well as a lower linoleic and arachidonic acid content. Comparison of each sample with one another reveals some over-all variability in fatty acid composition. Case 14, for instance, exhibits a higher content of palmitic acid and an unusually low content of arachidonic acid for each of the three lipoprotein fractions. From these preliminary data it would appear also that the highest level of the post arachidonic acid group is to be found in the phospholipid moiety of the S_f° 0−20 class lipoproteins. This may be the result of the relatively high content of sphingomyelins in the S_f° 0−20 lipoprotein class. Preliminary work[16] has

shown isolated sphingomyelins to contain a high percentage of these fatty acids with elution times longer than arachidonic acid.

COMMENTS

Previous chemical composition studies[6,17,18] have established the basic chemical composition for each of the lipids of the major serum lipoprotein classes. All of the major classes of lipids and some protein are found as part of the chemical structure of each lipoprotein class. However, each class is characterized by dominance of one or more lipid class. Thus, the s_f° $20-10^5$ lipoproteins consist largely of glycerides, the s_f° $0-20$ is composed of primarily unesterified cholesterol and esterified cholesterol, and the HDL-2 and 3 lipoproteins contain the highest content of phospholipid and unesterified fatty acids. Thus, fatty acid studies on the principal lipid moieties of each lipoprotein class reveal an additional and more detailed aspect of the chemical composition and structure of the serum lipoproteins. These preliminary fatty acid compositions studies indicate that for a given serum sample the fatty acid composition of each of the major lipid classes (cholesteryl esters, glycerides and phospholipids) are broadly similar with each of the three major lipoprotein fractions with potentially one exception. This exception is that the fatty acid distribution of the cholesteryl ester moiety of the s_f° $20-10^5$ class appears to contain an elevated level of palmitic and oleic as well as a lower linoleic acid content relative to that in the cholesteryl ester moiety of the other two major lipoprotein classes. The association of the s_f° $20-10^5$ lipoprotein fraction with the early phases of fat absorption and transport may be responsible, in part, for these chemical differences. Thus, cholesteryl esters in the early phase of transport in the blood stream may have a different fatty acid distribution from the cholesteryl esters that persist for longer periods of time in the bloodstream as part of the lipid moieties of the s_f° $0-20$ and HDL-2 and 3 lipoproteins. These findings suggest the potential importance of considering and studying in greater detail the macromolecular form in which a given class of lipids such as cholesteryl esters is transported in the blood stream.

SUMMARY

Gas-liquid chromatographic studies of each of the principal fatty acid-containing serum lipid fractions (cholesteryl esters, glycerides, unesterified fatty acids and phospholipids) reveal a relatively characteristic fatty acid pattern. Some variability, particularly in the cholesteryl ester fatty acid distribution, exists from person to person. In any given subject, the principal fatty acid-containing lipid fractions isolated from each of the major serum lipoprotein classes appear similar with one exception. Our preliminary data suggest the cholesteryl ester fraction of the s_f° $20-10^5$ lipoproteins contain a higher content of oleic and palmitic acid as well as a lower content of linoleic acid relative to the cholesteryl ester fractions obtained from the s_f° 0–20 and HDL-2 and 3 lipoprotein classes.

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Studies on the Characterization of Human Serum Lipoproteins Separated by Ultracentrifugation in a Density Gradient

I. Serum Lipoproteins in Normal, Hyperthyroid and Hypercholesterolemic Subjects

DAVID G. CORNWELL, PH.D.,* FRED A. KRUGER, M.D.,† GEORGE J. HAMWI, M.D.‡ AND J. B. BROWN, PH.D.§

HUMAN SERUM lipoproteins have been isolated and characterized by experimental procedures utilizing several different properties of lipoprotein molecules including their solubility,[1,2] precipitation with sulfated polysaccharides,[3–6] electrophoretic mobility[7,8] and density.[3,9–16] While the different methods for lipoprotein isolation have distinct advantages and limitations,[12,16–18] differential centrifugation in solutions of homogeneous density[12–14,19,20] and centrifugation in density gradients[3,15] are two experimental procedures which can be employed readily in studies in which the isolation, quantitative estimation and chemical analysis of lipoprotein fractions are all required. Density gradient technics have been used in the purification of specific lipoprotein fractions for chemical[3,15,21–24] and immunochemical[25] studies, and the separation of serum lipoprotein fractions in the investigation of lipoprotein metabolism.[22,26,27] However, density gradient technics have not been used to characterize the lipoprotein fractions and measure the distribution of lipids within the lipoprotein fractions of subjects with hypercholesterolemia and hyperlipemia.

In the present investigation, the serum lipoproteins of normal subjects were separated by centrifugation in a density gradient and characterized by their cholesterol, phospholipid and fatty acid ester content. Lipoprotein fractions isolated in the density gradient were refractionated by centrifugal flotation at different homogeneous densities in order to assess the purity of the lipoprotein fractions and study the homogeneity of the fractions by isolating and analyzing lipoprotein subfractions. The serum lipoproteins of euthyroid hypercholesterolemic, hypothyroid and hyperthyroid subjects were then isolated by the same procedures and compared with serum lipoproteins of normal subjects. Serum lipoproteins were isolated and characterized during the administration of thyroid hormones (desiccated thyroid, triiodothyronine and triiodothyroacetic acid) to hypothyroid and euthyroid hypercholesterolemic subjects. The isolation and analysis of serum lipoproteins in hyperlipemic subjects is described in a subsequent paper.[28]

From the Departments of Physiological Chemistry and Medicine, The Ohio State University, Columbus, Ohio.

* Associate Professor of Physiological Chemistry; † Associate Professor; ‡ Professor of Medicine and Chief, Division of Endocrinology and Metabolism; § Professor and Chairman, Department of Physiological Chemistry.

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METHODS

Experimental Subjects

Normal subjects included laboratory personnel, students and patients in the University Hospital with no evidence of either abnormal lipid metabolism or thyroid function. Thyroid function was estimated from basal metabolic rate, serum protein bound iodine, and radioactive iodide uptake. Normal ranges for euthyroid subjects in this laboratory are basal metabolic rate, plus 3 to minus 13; serum protein bound iodine 3 to 8 μg. per cent and radioactive iodine uptake 17 to 37 per cent.

Lipoprotein Fractionation

Serum lipoproteins were separated into their specific density classes by ultracentrifugal flotation in a saline density gradient.[3,15,21,22,27] Samples of venous blood were collected and allowed to clot for three hours at 4°c. Serum was separated from the fibrin and formed elements by centrifugation at 500 × g for thirty minutes. The initial centrifugation was performed in a low centrifugal field to minimize separation and concentration of the chylomicron fraction. Four milliliter of serum were placed in a 13.5 ml. lusteroid centrifuge tube. A saline solution, 4.5 ml. of 0.15 M sodium chloride,* was layered over the serum and the tube centrifuged for thirty minutes at 9,300 × g in a Spinco Model L ultracentrifuge. Chylomicrons, which formed a turbid band at the top of the saline layer, were separated from the serum with a tube cutter. Examination of the serum and the chylomicron fraction by dark-field microscopy indicated that the visible chylomicron particles were concentrated in the chylomicron layer. The infranatant serum was layered over 5.5 ml. of 2.0 M sodium chloride in a 13.5 ml. lusteroid centrifuge tube. This tube was filled with 0.15 M sodium chloride and centrifuged from eighteen to twenty-two hours at 100,000 × g. The S_f 10–400 lipoproteins† were concentrated in a turbid band at the top of the centrifuge tube, the S_f 3–9 lipoproteins formed an orange-yellow band in the center of the tube, while high density lipoproteins and other serum proteins sedimented to the bottom of the tube. These lipoprotein bands were separated with a tube cutter.

In some experiments, the S_f 10–400 and S_f 3–9 lipoprotein fractions obtained in the density gradient procedure were refractionated by a second centrifugation procedure. The S_f 10–400 lipoprotein fraction was placed in a 13.5 ml. centrifuge tube, the tube filled by layering 0.15 M sodium chloride over the lipoprotein fraction, and centrifuged from eighteen to twenty-two hours at 100,000 × g. Two S_f 10–400 lipoprotein subfractions were obtained. One fraction, S_f 10–400 ($D < 1.005$), floated to the top of the tube and was separated together with the solution in upper fourth of the tube. The other fraction, S_f 10–400 ($D > 1.005$), sedimented and was separated together with the other three-fourths of the tube contents. The S_f 3–9 lipoprotein fraction was placed in a 13.5 ml. centrifuge tube and adjusted to a density of 1.07 ± 0.003 with a sodium chloride-potassium bromide stock solution.[12] The tube was filled by layering 1.65 M sodium chloride (density 1.063) over the lipoprotein fraction and centrifuged from eighteen to twenty-two hours at 100,000 × g. Two lipoprotein fractions were obtained. One fraction, S_f 3–9 (A), floated to the top of the tube and was separated together with the solution in the upper fourth of the tube. The other fraction, High Density (A), sedimented to the bottom of the tube and was separated together with the other three-fourths of the tube contents. This sedimenting fraction was designated "High Density (A)" since the chemical analysis indicated that the fraction was composed of high density lipoproteins (see Results).

Extraction and Analysis of Lipids

Serum and lipoprotein fractions were transferred to volumetric flasks for lipid extraction.

* All sodium chloride solutions used in the isolation of serum lipoproteins contained 0.1 gm. per liter of the disodium salt of ethylenediaminetetraacetic acid adjusted to pH 7.0 ± 0.2 with 1 N sodium hydroxide.[3]

† Low density lipoprotein fractions are designated as S_f 10–400 and S_f 3–9 lipoproteins, the S_f distributions obtained in the analysis of these lipoprotein fractions by analytical ultracentrifugation.[3]

TABLE
The Distribution of Cholesterol and Phospholipid

	Serum			Chylomicron		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
						Normal Subjects
Mean	204.0	233.0	0.88	4.7	5.6	0.88
Standard deviation	27.6	33.3	0.07	1.8	2.2	0.28
						Hyperthyroid
Mean	165.0	212.0	0.78	3.7	5.0	0.75
Standard deviation	31.0	37.0	0.09	3.3	3.5	0.27
t	4.30†	1.93	4.13†	1.31	0.70	1.48
						Hypercholesterolemic
						Hypothyroid
Mean	427.0	403	1.05	8.2	7.6	1.05
Standard deviation	109.0	76.6	0.14	5.6	4.5	0.27
t	−10.8†	−10.8†	−5.78†	−3.15†	−2.05‡	−2.08‡
						Euthyroid
Mean	455.0	408.0	1.11	6.5	5.9	1.20
Standard deviation	142.0	104.0	0.15	1.9	2.4	0.32
t	−9.44†	−8.35†	−6.82†	−2.87†	−0.39	−3.20†

* Cholesterol: phospholipid ratio.
† Significant at 1 per cent level.
‡ Significant at 5 per cent level.

The chylomicron, S_f 10–400, and high density lipoprotein fractions were extracted in 50 ml. volumetric flasks, while the S_f 3–9 lipoprotein fraction was extracted in a 100 ml. volumetric flask. In the analysis of serums from hypercholesterolemic subjects, the S_f 3–9 lipoprotein fraction was extracted in a 250 ml. volumetric flask. Lipids were extracted with hot alcohol-ether (3:1, v/v), diluted to volume, and filtered. Lipid phosphorus, cholesterol and total fatty acid esters were then estimated with aliquots of the lipid extract. Phosphorus was determined by the method of Lowry et al.[29] and phospholipid estimated by multiplying the phosphorus concentration by 25. Total cholesterol was determined by the method of Abell et al.[30] The cholesterol used in standard solutions was first purified by the bromination procedure of Fieser.[31] Total fatty acid esters were determined by the method of Stern and Shapiro.[32]

RESULTS

Isolation and Analysis of Serum Lipoprotein Fractions

Lipoprotein fractions were isolated from normal, euthyroid hypercholesterolemic, hyperthyroid and hypothyroid subjects. Serum and the lipoprotein fractions were then analyzed for cholesterol, phospholipid and fatty acid ester (Tables I and II). Mean percentage recoveries for the different lipids were cholesterol 99 per cent, phospholipid 98 per cent and fatty acid esters 92 per cent.

The mean concentration and standard devia-

I
in Serum and Serum Lipoprotein Fractions

S_f 10–400			S_f 3–9			High Density		
Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
(Thirty-One)								
21.4	28.7	0.76	139.0	114.0	1.23	38.1	79.4	0.49
9.4	13.5	0.12	23.4	23.2	0.10	9.5	20.3	0.07
Subjects (Fifteen)								
18.6	25.2	0.78	109.0	109.0	1.02	30.8	66.3	0.46
9.5	14.9	0.13	25.0	28.7	0.14	8.2	13.7	0.06
0.94	0.79	...	3.98†	0.63	5.85†	2.54‡	2.24‡	...
Subjects (Thirty-Four)								
(Twenty-One)								
50.4	54.6	0.91	324.0	234.0	1.33	40.0	79.9	0.51
22.0	18.7	0.17	99.5	66.7	0.16	12.2	24.2	0.08
−6.53†	−5.79†	−3.79†	−9.89†	−9.95†	−2.79†	−0.63	...	−0.95
(Thirteen)								
44.7	49.0	0.98	334.0	245.0	1.36	42.6	78.6	0.54
27.0	35.7	0.25	115.0	72.3	0.17	9.9	14.4	0.08
−4.09†	−2.65‡	−3.79†	−8.74†	−8.79†	−3.03†	−1.36	...	−0.68

tion for serum lipids in normal subjects is similar to that found by other investigators.[12,33,34] Serum cholesterol is significantly decreased in hyperthyroid subjects while serum phospholipid is decreased to a lesser extent. The cholesterol: phospholipid ratio is lowered correspondingly. Similar results have been obtained by Boyd and Connell[35] and Foldes and Murphy.[36] However, Peters and Man[37] were unable to correlate decreased serum cholesterol with hyperthyroidism. Both cholesterol and phospholipid are elevated significantly in the serum of hypothyroid subjects. The cholesterol is elevated more than the phospholipid and the cholesterol: phospholipid ratio increased correspondingly. These results confirm many observations on serum lipids in hypothyroid subjects.[34–37] Fatty acid esters are elevated in hypothyroid subjects;* however, the fatty acid ester: cholesterol ratio is unchanged. The composition of serum lipids in euthyroid hypercholesterolemic subjects with idiopathic hypercholesterolemia and atherosclerosis, and also in some subjects with nephroses, is the same as the composition of serum lipids in hypothyroid subjects. Cholesterol, phospholipid and fatty acid esters* are elevated. The cholesterol: phospholipid ratio is elevated and the fatty acid ester: cholesterol ratio is unchanged. These results correspond to other observations on serum lipids in euthyroid hypercholesterolemic subjects.[34,38]

* Data from hypothyroid and euthyroid hypercholesterolemic subjects were pooled for a statistical analysis of the fatty acid ester distribution in serum and between lipoprotein fractions.

TABLE II
The Distribution of Fatty Acid Ester (FAE) in Serum and Serum Lipoprotein Fractions

	Serum		Chylomicron		S_f 10–400		S_f 3–9		High Density	
	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*
Normal Subjects (Eleven)										
Mean	0.89	0.49	0.05	1.04	0.23	1.13	0.40	0.33	0.14	0.48
Standard deviation	0.22	0.15	0.05	1.03	0.14	0.22	0.08	0.08	0.03	0.12
Hypercholesterolemic Subjects (Eighteen)										
Mean	1.79	0.41	0.05	0.75	0.39	0.79	1.02	0.29	0.19	0.61
Standard deviation	0.37	0.08	0.03	0.38	0.12	0.17	0.38	0.06	0.05	0.15
t	−7.36†	1.90	...	0.67	−3.29†	4.52†	−5.35†	1.55	−2.99†	−2.46‡

* Fatty acid ester (mEq.) × 100:cholesterol (mg.) ratio.
† Significant at 1 per cent level.
‡ Significant at 5 per cent level.

Chylomicron fractions separated by the density gradient procedure contain from 2 to 3 per cent of the total serum cholesterol and phospholipid (Table I). The cholesterol and phospholipid content and the cholesterol:phospholipid ratio of the chylomicron fraction appear to reflect those of the serum. However, the fatty acid ester:cholesterol ratio for the chylomicron fraction is significantly greater than this ratio for whole serum (Table II). The particles in serum visible by dark-field microscopy are concentrated in this fraction. Lipid distributions, fatty acid ester:cholesterol ratios, and dark-field examination suggest that the chylomicron fraction is contaminated with small amounts of serum, and thus that differences in the cholesterol and phospholipid content of chylomicron fractions reflect differences in serum lipid concentrations. Fatty acid ester analyses are similar for chylomicron fractions from normal and hypercholesterolemic groups whereas chylomicron fractions isolated from the serum of hyperlipemic subjects are often elevated in lipid content and altered in lipid composition.[28]

The S_f 10–400 lipoprotein fraction (Tables I and II) separated from normal serum by the density gradient procedure has the same lipid content and cholesterol:phospholipid ratio as S_f 10–400 lipoproteins isolated by dextran sulfate precipitation and purified in a density gradient,[3,17] and comparable $D < 1.019$ and α_2-lipoprotein fractions isolated by ultracentrifugation and electrophoresis, respectively.[12,13,16,17] Two S_f 10–400 lipoprotein subfractions, S_f 10–400 ($D < 1.005$) and S_f 10–400 ($D > 1.005$), were prepared by the recentrifugation of the S_f 10–400 lipoprotein fraction at density 1.005 (Table III). The denser lipoprotein fraction, S_f 10–400 ($D > 1.005$), has a higher cholesterol:phospholipid ratio. Bragdon et al.[13] have suggested that this fraction may represent the S_f 10–17 lipoproteins.

No significant differences were found between the S_f 10–400 lipoproteins of normal and hyperthyroid subjects (Table I). Hypothyroid and euthyroid hypercholesterolemic subjects show significant elevations in cholesterol and phospholipid content and cholesterol:phospholipid ratio of their S_f 10–400 lipoprotein fractions (Table I). Fatty acid esters are elevated in the S_f 10–400 lipoprotein fractions of hypercholesterolemic subjects while the fatty acid ester:cholesterol ratios are decreased (Table II). Lipid distributions between the

TABLE III
The Distribution of Cholesterol and Phospholipid in S_f 10–400 Lipoprotein Subfractions

	S_f 10–400			S_f 10–400 (D < 1.005)			S_f 10–400 (D > 1.005)		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Five)									
Mean	27.8	35.6	0.78	15.2	22.5	0.68	9.8	10.6	0.92
Standard deviation	8.9	10.7	0.06	5.4	7.9	0.06	1.8	1.6	0.13
Hypercholesterolemic Subjects (Four)									
Mean	51.1	60.0	0.87	29.4	43.6	0.70	19.5	19.2	1.05
Standard deviation	20.3	24.4	0.10	13.6	21.8	0.07	8.8	8.6	0.22

* Cholesterol: phospholipid ratio.

S_f 10–400 lipoprotein subfractions of hypercholesterolemic subjects are similar to the normal distribution, although the S_f 10–400 (D > 1.005) fraction of hypercholesterolemic subjects appears to have a higher cholesterol: phospholipid ratio (Table III). Since the cholesterol: phospholipid ratio increases and the triglyceride content decreases in the transition from higher to lower S_f lipoprotein fractions,[3,13,17,24] the changes in the cholesterol: phospholipid and fatty acid ester: cholesterol ratios both suggest that S_f 10–20 or S_f 10–100 lipoproteins are more abundant in the S_f 10–400 lipoproteins of hypercholesterolemic subjects. An elevated S_f 12–20 lipoprotein fraction has been found in the serum of hypothyroid subjects by analytical ultracentrifugation.[39,40] We have found that when the S_f 10–400 lipoprotein fraction is markedly elevated there are often alterations in the cholesterol: phospholipid ratio and lipid distribution between subfractions.[28]

The S_f 3–9 lipoprotein fraction separated from normal serum by the density gradient procedure has a lipid content similar to comparable lipoprotein fractions isolated by Cohn fractionation, ultracentrifugation[3,12,14,16] or electrophoresis.[17,24] The mean cholesterol: phospholipid ratios, 1.23 (Table I) and 1.27 (Table IV), are in the lower range of cholesterol: phospholipid ratios, 1.20 to 1.66, reported in other studies. When a purified lipoprotein fraction, S_f 3–9 (A), is isolated by recentrifugation (Table IV), the cholesterol: phospholipid ratio, 1.41, is the same as this ratio for repurified or carefully isolated S_f 3–9 lipoprotein fractions.[3,12,13] Recentrifugation demonstrates further that the S_f 3–9 lipoprotein fraction (Table IV) is contaminated with high density lipoproteins, High Density (A) (Table V), which account for 7.6 to 12 per cent and 17.6 to 20.5 per cent of the S_f 3–9 lipoprotein cholesterol and phospholipid, respectively.*

The mean concentration of serum cholesterol in the S_f 3–9 lipoprotein fractions isolated from hyperthyroid subjects is significantly lower than normal while the decrease in phospholipid content is less significant (Tables I and IV). The cholesterol: phospholipid ratio is lowered correspondingly (Table I). However, the cholesterol phospholipid ratio for repurified S_f 3–9 (A) lipoproteins isolated from hyperthyroid subjects is in the normal range (Table

* The S_f 3–9 lipoprotein fraction can be separated completely from the high density lipoproteins by increasing the volume of 2 M sodium chloride in the gradient tube. The gradient described in this study was chosen specifically to enhance the separation of S_f 10–400 and S_f 3–9 lipoprotein fractions, since major variations in the serum lipids generally reflect alterations in the composition and S_f distribution of the S_f 10–400 lipoprotein fraction.

TABLE IV

The Distribution of Cholesterol and Phospholipid in S_f 3–9 and Repurified S_f 3–9 (A) Lipoprotein Fractions

	S_f 3–9			S_f 3–9 (A)		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Eight)						
Mean	134.0	106.0	1.27	118.0	83.7	1.41
Standard deviation	25.6	22.3	0.06	25.9	17.0	0.10
Hyperthyroid Subjects (Four)						
Mean	87.9	78.6	1.11	73.6	55.3	1.32
Standard deviation	20.8	10.9	0.15	19.5	8.6	0.16
Hypercholesterolemic Subjects (Five)						
Mean	282.0	203.0	1.39	248.0	174.0	1.43
Standard deviation	69.9	40.3	0.18	51.0	26.6	0.13

* Cholesterol:phospholipid ratio.

IV). It can be seen in Table V that the high density lipoprotein contamination, High Density (A), is similar in amount for normal and hyperthyroid groups. These results suggest that S_f 3–9 lipoproteins in normal and hyperthyroid subjects have the same composition but that high density lipoprotein contamination has a greater effect on the cholesterol:phospholipid ratio because the S_f 3–9 lipoprotein fraction is present in a lower concentration in hyperthyroid subjects.

The S_f 3–9 lipoprotein fractions isolated from hypothyroid and euthyroid hypercholesterolemic subjects are markedly elevated (Tables I and IV). Mean cholesterol, phospholipid and cholesterol:phospholipid ratios are all elevated significantly above normal. Nevertheless, the cholesterol:phospholipid ratio for repurified S_f 3–9 (A) lipoproteins isolated from hypercholesterolemic subjects corresponds to the normal mean ratio (Table IV). Since the high density contamination, High Density (A), is similar in amount for normal and hypercholesterolemic groups (Table V), contamination has less effect on the cholesterol:phospholipid ratio in the elevated S_f 3–9 lipoprotein fraction. An elevation in the low density lipoprotein fraction, specifically the S_f 3–9 or β-lipoproteins,[3] in hypothyroid and euthyroid hypercholesterolemic subjects has been described in other studies using analytical ultracentrifugation[39–41] and paper electrophoresis.[42–44] Fatty acid esters are elevated in the S_f 3–9 lipoprotein fraction of hypercholesterolemic subjects (Table II), however, the fatty acid:cholesterol ratio corresponds to the normal ratio. The normal cholesterol:phospholipid ratio for the S_f 3–9 (A) lipoprotein fraction and the normal fatty acid ester:cholesterol ratio both suggest that the S_f 3–9 lipoprotein fraction isolated from hypercholesterolemic subjects is similar in composition and differs only in amount from the S_f 3–9 lipoprotein fraction in normal subjects. Jones et al.[39] have suggested from analytical ultracentrifugation studies that the S_f 3–9 lipoproteins of hypothyroid subjects differ from the S_f 3–9 lipoproteins of atherosclerotic hypercholesterolemic subjects. This observation was not confirmed in the present study.

The high density lipoprotein fraction isolated from normal subjects by the density gradient procedure (Tables I and II) is similar in composition to the high density lipoproteins iso-

TABLE V
The Distribution of Cholesterol and Phospholipid in the High Density Lipoproteins

	High Density			High Density (A)			High Density (T)		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Eight)									
Mean	41.5	94.0	0.44	10.2	18.7	0.56	51.7	113	0.46
Standard deviation	7.5	13.8	0.05	5.7	11.5	0.09	9.8	19.9	0.05
Hyperthyroid Subjects (Four)									
Mean	31.8	70.3	0.45	8.3	17.6	0.48	40.1	87.7	0.46
Standard deviation	8.1	13.6	0.04	2.1	5.4	0.06	9.6	16.4	0.04
Hypercholesterolemic Subjects (Five)									
Mean	40.2	85.3	0.47	13.1	20.8	0.64	53.3	106	0.50
Standard deviation	18.0	33.8	0.04	7.8	15.0	0.22	25.1	48.3	0.07

* Cholesterol:phospholipid ratio.

lated from normal subjects in other studies.[2,12–14,16,17,20] However, the concentration of serum lipids in this fraction separated by the density gradient procedure is lower than concentrations reported in the studies using other isolation procedures. When the high density lipoprotein contaminant in the S_f 3–9 lipoprotein fraction, High Density (A), is added to the high density fraction (Table v), the total fraction, High Density (T), has a lipid concentration similar to that reported in other studies.[2,12–14,16,17,20] High density lipoproteins isolated from hyperthyroid subjects have the same cholesterol:phospholipid ratio but a somewhat lower serum concentration than high density lipoproteins isolated from normal subjects (Tables I and v). High density lipoproteins isolated from hypothyroid and euthyroid hypercholesterolemic subjects have the same concentration and cholesterol:phospholipid ratio as the normal group (Tables I and v). Jones et al.[39] have also reported that the concentration of high density lipoproteins is not altered significantly in hypothyroid and euthyroid hypercholesterolemic subjects. Fatty acid esters and the fatty acid ester:cholesterol ratio are elevated in the high density lipoprotein fraction of hypercholesterolemic subjects (Table II). Although this observation might suggest that a difference exists between the composition of high density lipoproteins in normal and hypercholesterolemic subjects, a corresponding difference for the cholesterol:phospholipid ratios was not observed (Tables I and v).

The Effect of Thyroid Hormones on the Serum Lipid Concentration and Lipoprotein Distribution in Hypercholesterolemic Subjects

Thyroid hormones (desiccated thyroid, triiodothyronine and triiodothyroacetic acid) were administered to hypothyroid and euthyroid hypercholesterolemic subjects. The clinical diagnosis and laboratory evaluation of representative subjects from each group and two hyperthyroid hypercholesterolemic subjects before and after I^{131} thyroidectomy is summarized in Table VI. Serum

TABLE VI

The Clinical Diagnosis and Laboratory Evaluation of Thyroid Function in Experimental Subjects

Subject, Age (yr.) and Sex	Date	Protein Bound Iodine (μg. %)	Radioactive Iodine Uptake (%)	Basal Metabolic Rate (%)	Diagnosis
			Hypothyroid Subjects		
J. S., 19, M	5/7/57	1.8	2	−35	Juvenile myxedema
H. R., 66, F	11/7/57	2.3	6	−32	Hypothyroid (I^{131} thyroidectomy)
G. C., 46, F	1/26/58	3.0	..	−17	Spontaneous hypothyroidism
D. M., 47, F	5/7/58	2.4	2	−39	Spontaneous hypothyroidism
			Euthyroid Subjects		
G. G., 37, M	6/5/58	5.9	..	...	Idiopathic hypercholesterolemia, arteriosclerosis, angina
A. W., 41, M	5/13/57	4.3	9	...	Idiopathic hypercholesterolemia
C. K., 30, F	9/3/56	6.0	47	3	Nephrotic phase of chronic glomerulonephritis
			Hyperthyroid Subjects		
H. S., 53, F	10/15/56	...	80	17	Diffuse toxic goiter, arteriosclerosis, angina
	3/12/58	6.0	18	−2	Euthyroid (I^{131} thyroidectomy)
E. H., 65, F	3/31/58	9.3	58	...	Nodular toxic goiter, arteriosclerosis, myocardial infarction
	7/6/58	4.5	20	...	Euthyroid (I^{131} thyroidectomy)

lipid concentrations and lipoprotein distributions were studied in these subjects before and during therapy.

Hypothyroid Subjects. The four hypothyroid subjects described in Table VI have initial serum lipid concentrations and lipoprotein distributions typical of hypothyroidism (Table VII). Serum cholesterol, phospholipid and the cholesterol:phospholipid ratio are all elevated. The chylomicron fractions are normal. Lipid concentrations and cholesterol:phospholipid ratios are elevated in the S_f 10–400 lipoprotein fractions of three subjects. The S_f 3–9 lipoprotein fraction is significantly elevated in the four subjects, while the high density lipoprotein fraction is normal.

Triiodothyronine, desiccated thyroid and triiodothyroacetic acid all reverse the serum lipid elevation and correct the abnormal lipoprotein distribution (Table VII). During therapy there is a rapid decrease in the concentration of serum cholesterol and phospholipid, and in the cholesterol:phospholipid ratio. The S_f 10–400 lipoprotein fractions of two subjects (J. S. and H. R.) are lowered to a normal level. The S_f 10–400 lipoprotein fraction of one subject (G. C.) was at a normal level before and during therapy, while the S_f 10–400 lipoprotein fraction of the fourth subject (D. M.) was lowered initially but then reverted to an elevated level. The serum lipids of this subject were the least well controlled by therapy. The four subjects all demonstrate a marked decrease in the concentration of the S_f 3–9 lipoprotein fraction. Alterations in the concentration of high density lipoproteins are less significant in magnitude. The high density lipoprotein fraction is lowered in three subjects (J. S., H. R., and G. C.) and elevated in one subject (D. M.), during therapy. These studies together with distribution studies on ten additional subjects during therapy suggest

TABLE VII

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of Hypothyroid Subjects During Therapy

Date	Serum			Chylomicron			S_f 10–400			S_f 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Subject J. S. (Triiodothyroacetic acid, 2 mg.)															
5/10/57	359	340	1.06	...	...	...	...	...	...	...	...	...	...	...	...
5/15/57	349	322	1.08	4.8	4.5	1.07	41.7	37.0	1.13	314	198	1.59	44.7	81.3	0.55
5/17/57	284	266	1.07	13.5	9.8	1.38	33.8	27.5	1.23	236	158	1.49	46.1	73.3	0.63
5/23/57	243	275	0.88	12.1	10.0	1.21	37.0	37.5	0.99	173	140	1.24	41.6	87.5	0.48
5/27/57	231	225	1.03	9.3	8.9	1.04	20.2	25.5	0.79	167	111	1.50	40.8	77.5	0.53
6/10/57	230	299	0.74	8.9	16.1	0.55	14.8	25.0	0.59	169	158	1.07	34.3	86.5	0.40
Subject H. R. (Desiccated thyroid, 1.5 gr.)															
11/22/57	435	430	1.01	...	...	...	44.0	42.5	1.04	359	244	1.47	48.0	97.5	0.49
12/6/57	304	321	0.95	4.2	3.8	1.11	17.0	22.5	0.76	230	201	1.14	40.4	82.5	0.49
6/26/58	229	263	0.87	2.9	3.8	0.76	15.2	18.8	0.81	164	148	1.11	35.3	71.5	0.49
Subject G. C. (Triiodothyronine, 75 μg.)															
1/29/58	411	450	0.91	7.7	6.8	1.13	24.6	33.0	0.75	328	268	1.22	53.9	111	0.49
4/5/58	291	344	0.85	4.4	6.0	0.73	22.8	30.2	0.75	235	201	1.17	38.2	87.0	0.44
7/25/58	258	298	0.86	4.4	5.3	0.83	21.5	24.0	0.90	194	172	1.13	35.8	82.8	0.43
10/14/58	259	301	0.86	4.8	6.8	0.71	25.4	30.3	0.84	192	160	1.20	39.2	96.8	0.40
Subject D. M. (Triiodothyronine, 50 μg.)															
5/9/58	655	600	1.09	6.7	6.2	1.08	37.4	44.5	0.84	564	453	1.25	29.6	79.3	0.37
5/19/58	531	425	1.25	6.2	5.2	1.19	12.4	12.5	0.99	511	331	1.54	24.3	56.3	0.43
5/23/58	452	450	1.00	5.4	5.5	0.98	23.1	23.8	0.97	412	350	1.18	21.6	57.5	0.38
6/16/58	428	355	1.21	6.3	5.0	1.26	30.7	26.8	1.15	365	249	1.47	25.1	57.0	0.44
9/3/58	396	376	1.05	8.8	15.0	0.59	61.1	61.0	1.00	287	238	1.21	26.3	72.3	0.36
4/8/59	393	385	1.02	5.6	8.1	0.69	44.6	50.9	0.88	295	224	1.32	38.4	107	0.36

* Cholesterol : phospholipid ratio.

that no significant and consistent alteration in the high density lipoprotein fraction of hypothyroid subjects can be attributed to thyroid therapy.

The two lipoprotein fractions elevated in hypothyroid subjects, S_f 10–400 and S_f 3–9 lipoproteins (Tables I through IV), are the lipoprotein fractions altered by thyroid hormones (Table VII). A decrease in serum lipids, the serum cholesterol : phospholipid ratio and concentration of the S_f 3–9 or β-lipoprotein fraction in hypothyroid subjects during thyroid therapy has been confirmed in many other investigations.[34,39–43]

Euthyroid Hypercholesterolemic Subjects. The three euthyroid hypercholesterolemic subjects described in Table VI have serum lipid concentrations and lipoprotein distributions typical of the hypercholesterolemic group (Table VIII). These subjects were chosen to serve as examples of triiodothyroacetic acid, desiccated thyroid and triiodothyronine therapy in euthyroid hypercholesterolemic subjects.

Triiodothyroacetic acid lowered serum lipids in one subject (G. G.). The concentration of the S_f 3–9 lipoprotein fraction was lowered correspondingly; however, no significant decrease was observed in the S_f 10–400 lipoprotein fraction. The high density lipoprotein fraction appeared to decrease in concentration although the high density lipoprotein changes during thyroid administration

TABLE VIII

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of Euthyroid Subjects with Hypercholesterolemia During Therapy

Date	Serum			Chylomicron			Sf 10–400			Sf 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Subject G. G.															
Triiodothyroacetic acid, 2 mg.															
5/2/57	559	447	1.25	8.8	6.0	1.47	69.5	53.3	1.30	415	281	1.48	48.2	72.9	0.66
5/6/57	568	486	1.17	14.7	11.5	1.28	78.1	84.6	0.92	445	285	1.56	29.2	77.3	0.38
5/10/57	564	444	1.27	17.0	12.3	1.38	110	91.9	1.20	481	297	1.62	32.5	75.3	0.43
5/16/57	513	450	1.14	11.5	8.7	1.32	35.5	46.8	0.76	425	287	1.48	48.6	85.0	0.57
5/20/57	474	394	1.20	24.2	15.5	1.56	38.0	39.4	0.96	433	300	1.44	22.9	51.3	0.45
6/10/57	483	435	1.11	10.2	9.8	1.04	51.6	53.8	0.96	378	280	1.35	27.1	72.5	0.37
6/18/57	454	335	1.36	31.7	27.1	1.17	33.5	45.0	0.74	341	238	1.43	28.0	62.5	0.45
6/20/57	436	334	1.31	37.4	22.9	1.63	40.7	36.2	1.12	314	208	1.51	26.5	58.8	0.45
8/7/57	474	419	1.13	...	...	...	...	...	...	...	...	...	...	...	...
12/10/57	484	446	1.09	7.3	7.0	1.04	43.9	44.3	0.99	387	310	1.25	27.5	69.3	0.40
1/16/58	497	444	1.12	4.2	3.8	1.11	36.1	43.0	0.84	447	325	1.38	22.9	56.8	0.40
No therapy															
6/4/58	634	553	1.15	7.3	5.3	1.38	60.9	55.0	1.11	522	385	1.36	28.0	68.8	0.41
Subject A. W.															
Desiccated thyroid, 3 gr.															
5/14/57	278	300	0.93	6.6	6.3	1.05	42.0	58.5	0.72	212	155	1.37	47.1	78.8	0.60
11/27/57	298	311	0.96	4.0	3.0	1.33	46.7	70.5	0.66	188	137	1.37	39.2	67.5	0.58
12/9/57	303	313	0.97	6.2	7.5	0.83	47.0	70.0	0.67	214	156	1.37	34.3	74.5	0.46
4/1/58	276	280	0.99	3.3	3.8	0.87	21.9	31.5	0.70	220	181	1.22	25.9	58.3	0.44
Subject C. K.															
No therapy															
6/1/57	454	414	1.10	8.3	5.4	1.54	48.9	52.5	0.93	319	239	1.33	52.9	96.5	0.55
8/6/57	424	379	1.12	...	...	...	...	...	...	...	...	...	...	...	...
8/13/57	415	360	1.15	25.0	14.3	1.75	45.1	32.5	1.06	341	198	1.72	35.1	71.3	0.49
9/20/57	505	454	1.11	25.8	22.8	1.13	61.7	69.6	0.89	389	267	1.46	51.4	95.1	0.54
12/11/57	466	488	0.95	...	...	...	56.7	54.0	1.05	363	303	1.20	37.1	83.8	0.44
Triiodothyronine, 50 μg.															
1/16/58	425	400	1.06	4.2	3.5	1.20	25.4	29.8	0.85	375	279	1.34	37.6	66.3	0.57
2/13/58	422	328	1.29	9.2	6.8	1.35	30.9	35.8	0.86	340	215	1.58	36.9	63.8	0.58
3/12/58	478	438	1.09	10.0	6.8	1.47	49.4	106	0.47	365	248	1.47	40.2	77.0	0.52
4/23/58	345	337	1.02	6.2	7.5	0.83	25.9	30.5	0.85	279	200	1.40	46.5	84.5	0.55
7/28/58	429	400	1.07	6.5	8.8	0.74	70.4	67.0	1.05	289	225	1.28	36.9	74.3	0.50

* Cholesterol: phospholipid ratio.

to euthyroid hypercholesterolemic subjects are not significant in magnitude or consistent in direction. Oliver and Boyd[44] administered triiodothyroacetic acid to atherosclerotic hypercholesterolemic subjects and found a decrease in serum cholesterol and β-lipoprotein cholesterol together with an increase in α-lipoproteins measured by paper electrophoresis. While similar results were obtained for serum and the S_f 3–9 or β-lipoproteins in the present study, the consistent increase in high density or α-lipoproteins was not confirmed.

The administration of desiccated thyroid appeared to lower the S_f 10–400 and high density lipoprotein fractions a small amount in one subject (Table VIII). This subject (A. W.)

TABLE IX

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of Two Hyperthyroid Subjects with Hypercholesterolemia

Date	Serum			Chylomicron			S_f 10–400			S_f 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Subject H. S.															
No therapy															
10/15/56	440	...	...	...	...	...	...	...	...	...	...	...	...	...	...
I^{131} thyroidectomy															
3/16/58	518	449	1.15	8.4	7.5	1.12	107	89.1	1.11	332	261	1.27	19.9	43.8	0.45
7/2/58	471	450	1.05	3.5	4.5	0.78	51.9	59.8	0.87	389	286	1.36	27.1	78.3	0.35
Subject E. H.															
No therapy															
3/31/58	396	...	...	...	...	...	...	...	...	...	...	...	...	...	...
I^{131} thyroidectomy															
7/8/58	372	368	1.01	6.5	7.4	0.88	25.9	37.8	0.69	305	232	1.31	48.0	102	0.47
9/22/58	440	396	1.11	6.2	6.8	0.91	28.3	32.5	0.87	317	246	1.29	57.1	104	0.55

* Cholesterol: phospholipid ratio.

was included to demonstrate that thyroid hormones have less effect on serum lipids when the subject has a mild hypercholesterolemia. Experiments on other subjects demonstrated that desiccated thyroid has an effect similar to triiodothyroacetic acid in subjects with a pronounced hypercholesterolemia. Strisower[40] found a similar correlation between the initial hypercholesterolemia and the response to thyroid therapy.

The third euthyroid hypercholesterolemic subject (C. K.) demonstrates both the variation in serum lipids and lipoproteins without therapy and the effect of triiodothyronine therapy on serum lipids and the lipoprotein fraction (Table VIII). The variation in serum lipids and lipoprotein distributions is considerable even when no therapy is employed. This emphasizes the difficulty in interpreting small changes in lipoprotein concentration unless the alterations are consistent in direction and reversed when therapy is discontinued. The alterations in the high density lipoprotein fraction (Tables VII and VIII) do not meet these criteria and cannot be interpreted with certainty. Serum lipids and the S_f 10–400 and S_f 3–9 lipoprotein fractions are lowered by the administration of triiodothyronine to this subject; however, the lipids and lipoproteins are not lowered and maintained at a normal level by therapy.

While the serum lipids and S_f 3–9 lipoproteins in euthyroid hypercholesterolemic subjects are lowered by the administration of thyroid hormones, the lipids are not lowered to normal levels and revert to the initial hypercholesterolemic state when therapy is continued for several months. This reversion in lipid concentration has been prevented by large doses of thyroid hormones[40]; however, a large thyroid dosage was not tolerated by subjects with angina and other euthyroid hypercholesterolemic subjects treated as outpatients in the present study.

Hyperthyroid Hypercholesterolemic Subjects. An elevated serum cholesterol was found in two hyperthyroid subjects. One subject (H. S.) had a diffuse toxic goiter and arteriosclerosis while the other subject (E. H.) had a nodular toxic goiter and arteriosclerosis (Table VI). Although lipoprotein distributions were not obtained initially, the hypercholesterolemic serum examined when the subjects were in a hyperthyroid state was not lactescent and probably represented hypercholesterolemia rather than hyperlipemia. Lipoprotein distributions (Table IX) were obtained after chemical thyroidectomy when the subjects were clinically euthyroid. The two subjects exhibited a typical hypercholesterolemia with elevated S_f 10–400 and S_f 3–9 lipoprotein fractions. These subjects were included in the study to emphasize that hypercholesterolemia may be found in hyperthyroid as well as hypothyroid and euthyroid subjects.

COMMENTS

Density gradient centrifugation is adequate for the separation of the major lipoprotein fractions of serum. A lipoprotein distribution is established which is related to the serum lipid concentration and pathological state of the subject. The density gradient is useful for the routine analysis of serum lipoproteins during therapy since a preliminary thirty minute centrifugation and only one eighteen to twenty-two hour centrifugation are required. However, the S_f 3–9 lipoprotein fraction isolated by this procedure is contaminated with a small but relatively consistent amount of the high density lipoprotein fraction. This contamination may represent from 5 to 10 per cent of the S_f 3–9 lipoprotein cholesterol depending upon the concentration of the S_f 3–9 lipoprotein fraction. When density gradient centrifugation is combined with a second centrifugation at a homogeneous density, a highly purified S_f 3–9 lipoprotein fraction is obtained. A second centrifugation may also be used to subfractionate the S_f 10–400 lipoprotein fraction into S_f 10–400 ($D < 1.005$) and S_f 10–400 ($D > 1.005$) fractions. Subfractionation is important in hyperlipemic states in which major alterations in the distribution of S_f 10–400 lipoproteins occur.[28] Thus the density gradient can be used both to establish a lipoprotein distribution and as the first step in subfractionation and purification of lipoprotein fractions.

The fatty acid ester analysis is a simple procedure which confirms and extends the information obtained by routine cholesterol and phospholipid analyses. Fatty acid ester: cholesterol ratios may be used to characterize serum and further establish the identity of lipoprotein fractions. While the fatty acid ester analysis is less specific than an analysis for neutral lipid or triglyceride, major alterations in the fatty acid ester:cholesterol ratio reflect alterations in neutral lipid and suggest alterations in lipoprotein fractions high in triglyceride.[28]

Total serum lipids are a summation of the concentration and composition of the different lipoprotein fractions present in serum. Serum cholesterol, phospholipid and fatty acid ester analyses, and the cholesterol:phospholipid and fatty acid ester:cholesterol ratios, are sufficient to define the type of lipoprotein distribution (S_f 10–400, S_f 3–9 or high density lipoprotein elevations). However, the composition of specific lipoprotein fractions or changes in the concentration of specific lipoprotein fractions cannot be estimated accurately by the analysis of whole serum. This is demonstrated in the studies on hypercholesterolemia.

The isolation and analysis of serum lipoproteins from hypercholesterolemic subjects show that hypercholesterolemia results from two specific alterations in serum lipoprotein fractions. The S_f 3–9 lipoprotein fraction is markedly elevated but not altered in composition since the cholesterol:phospholipid and fatty acid ester:cholesterol ratios are normal. The S_f 10–400 lipoprotein fraction is moderately elevated. Furthermore, the analysis of S_f 10–400 lipoprotein subfractions, the increase in the cholesterol:phospholipid ratio, and the decrease in the fatty acid ester:cholesterol ratio, all suggest that the elevation in S_f 10–400 lipoproteins is primarily in the S_f 10–20 range. The same lipoprotein distribution is observed in all hypercholesterolemic subjects regardless of their thyroid state.

Elevated serum lipids and lipoprotein fractions in hypothyroid subjects are controlled by the administration of thyroid hormones and the maintenance of subjects in a euthyroid state. The elevated S_f 10–400 and S_f 3–9 lipoprotein fractions are lowered to normal levels during therapy, whereas no significant alteration in high density lipoproteins occurs. These observations are consistent with present concepts of lipoprotein metabolism. Lipoprotein distribution studies show that the high density lipoprotein fraction is not elevated in hypercholesterolemia. Immunochemical studies have shown that the S_f 10–400 and S_f 3–9 lipoprotein fractions have related protein moieties which are distinct from the proteins in the high density lipoprotein fraction.[25] Several studies have shown that the S_f 10–400 and S_f 3–9 lipoprotein fractions are related metabolically as precursor and product and are independent of high density lipoprotein metabolism.[26,27]

While the administration of thyroid hormones lowers serum lipids and elevated lipoprotein fractions in euthyroid hypercholesterolemic subjects, serum lipids are not lowered and maintained at normal concentrations by thyroid therapy. Furthermore, hypercholesterolemia has been demonstrated in hyperthyroid subjects. These observations suggest that hypercholesterolemia may originate from several distinct metabolic defects even though the abnormal lipoprotein distributions that result are the same. Olson[45] has suggested that hypercholesterolemia originates either from a primary hepatic overproduction of cholesterol or the underutilization of cholesterol and relative overproduction. Both overproduction and underutilization result in hypercholesterolemia or an elevated body pool when cholesterol metabolism reaches a steady state. A number of experimental studies support this hypothesis. The half-life of plasma cholesterol is the same in normal subjects and hypercholesterolemic subjects with xanthoma tendinosum.[46,47] Since the body cholesterol pool is greatly enlarged in hypercholesterolemia, primary hepatic overproduction of cholesterol is suggested.[45] In hypothyroidism cholesterol synthesis is decreased[48]; however, decreased synthesis is more than compensated by decreased cholesterol catabolism[48–51] and this also results in an increased cholesterol pool.[45] The degradation of cholesterol to bile acids, a major catabolic pathway in cholesterol metabolism,[52] is decreased in hypothyroidism.[53] Thyroid hormones may be expected to increase the cholesterol to bile acid conversion; however, bile acid production is limited by a bile acid feed-back mechanism[52] which may ultimately limit the conversion process.[48] This feed-back mechanism may operate in hyperthyroidism when only a mild hypocholesterolemia develops. These studies on cholesterol metabolism suggest that thyroid hormones may be less effective in controlling hypercholesterolemia caused by primary hepatic overproduction than hypercholesterolemia caused by underutilization of cholesterol. The observations in the present investigation support this concept and suggest that thyroid hormone therapy is a questionable method for the control of hypercholesterolemia produced through the hepatic overproduction of cholesterol in euthyroid subjects.

SUMMARY

Ultracentrifugation in a density gradient is an adequate one-step procedure for the separation of human serum lipoproteins, although 5 to 10 per cent of the cholesterol in the S_f 3–9 lipoprotein fraction isolated by this method represents contamination with high density lipoproteins. The density gradient procedure may be combined with ultracentrifugation at homogeneous densities in order to purify the S_f 3–9 lipoprotein fraction and subfractionate the S_f 10–400 lipoproteins. Purification and subfractionation are necessary for the characterization of serum lipoprotein distributions.

A total fatty acid ester analysis (hydroxamic acid method) supplements cholesterol and phospholipid analyses on serum and serum lipoprotein fractions. Fatty acid ester:cholesterol ratios as well as cholesterol:phospholipid ratios may be used to characterize lipoprotein fractions and serum lipoprotein distributions. Serum lipid concentrations and the cholesterol:phospholipid and fatty acid ester:cholesterol

ratios reflect the concentration and composition of the constituent lipoproteins in serum. While the analysis of total serum lipids indicates the general nature of the lipoprotein pattern, the specific details of the lipoprotein pattern are obtained only by fractionation.

Hyperthyroid subjects exhibit moderate hypocholesterolemia, a significantly lowered S_f 3–9 lipoprotein fraction and a moderately lowered high density lipoprotein fraction. These lipoprotein fractions have a normal composition. Hypercholesterolemic subjects, whether hypothyroid, euthyroid or hyperthyroid, all have the same abnormal lipoprotein pattern. This is characterized by elevated serum cholesterol, phospholipid and fatty acid esters, an elevated cholesterol: phospholipid ratio, and a normal fatty acid ester:cholesterol ratio. The chylomicron and high density lipoprotein fractions have a normal concentration; the high density lipoprotein fraction may have a slightly elevated fatty acid ester content. The S_f 3–9 lipoprotein fraction has a normal composition but is significantly elevated in concentration. The S_f 10–400 lipoprotein fraction is moderately elevated and probably concentrated in the S_f 10–20 distribution range.

In hypothyroid subjects, hypercholesterolemia can be corrected by the administration of thyroid hormones. Elevated S_f 10–400 and S_f 3–9 lipoprotein fractions are lowered and maintained at normal levels during therapy. No significant alteration in the high density lipoprotein fraction occurs, an observation consistent with the hypothesis proposed for lipoprotein metabolic relationships. Hypercholesterolemia in euthyroid subjects is not adequately controlled by thyroid hormones. The alterations in cholesterol metabolism proposed as causal factors in hypercholesterolemia are summarized as (1) a primary hepatic overproduction of cholesterol (euthyroid hypercholesterolemia), and (2) underutilization of cholesterol (hypothyroid hypercholesterolemia). While thyroid hormones may increase the cholesterol to bile acid conversion, it is suggested that a bile acid feed-back mechanism prevents the adequate control of hypercholesterolemia observed in euthyroid subjects.

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Studies on the Characterization of Human Serum Lipoproteins Separated by Ultracentrifugation in a Density Gradient.

II. Serum Lipoproteins in Hyperlipemic Subjects

DAVID G. CORNWELL, PH.D.,* FRED A. KRUGER, M.D.,† GEORGE J. HAMWI, M.D.‡ AND J. B. BROWN, PH.D.§

ABNORMAL ELEVATIONS in serum lipids have been classified as hyperlipemia, hypercholesterolemia and hyperphospholipidemia according to the major lipid increment, triglyceride, cholesterol or phospholipid, in the serum.[1–6] Such a classification implies that the different lipid classes may vary independently, whereas in fact they do not. Thus phospholipids are increased in hypercholesterolemias, and cholesterol and phospholipids are elevated as well as triglycerides in hyperlipemias. It is now apparent that the various serum lipids are present as components of specific lipoproteins which can be separated on the basis of differences in physical and chemical properties.[2,3,5–13] Furthermore, these lipoproteins are characterized by their relative triglyceride, cholesterol (free and esterified) and phospholipid content. Thus the four major groups of lipoproteins as separated according to increasing density—chylomicrons, S_f 10–400, S_f 3–9 and high density lipoproteins—differ both quantitatively and qualitatively in their lipid content.[11,13]

Lipoprotein fractions in the serum of hyperlipemic subjects have been investigated by Cohn fractionation,[4,5,9] electrophoresis,[2,5,10] analytical ultracentrifugation,[7,8] differential centrifugation in solutions of homogeneous density[5,12] and centrifugation in a density gradient.[11] These studies demonstrate elevated low density lipoproteins, particularly the $S_f > 10$ fraction, in the serum of hyperlipemic subjects.

In the present investigation, the serum lipoproteins of hyperlipemic subjects were separated by centrifugation in a density gradient and refractionated by centrifugal flotation at different homogeneous densities.[13] The lipoprotein fractions were characterized by their cholesterol, phospholipid and fatty acid ester content.[13] Serum lipoproteins were isolated and characterized during the administration of insulin in the control of diabetic acidosis and during the administration of unsaturated fatty acids to hyperlipemic subjects.

METHODS

The clinical diagnosis of each hyperlipemic subject is given in Table I. Procedures used in the isolation and characterization of serum lipoprotein fractions are described in the pre-

From the Departments of Physiological Chemistry and Medicine, The Ohio State University, Columbus, Ohio.

* Associate Professor of Physiological Chemistry; †Associate Professor; ‡Professor of Medicine and Chief, Division of Endocrinology and Metabolism; §Professor and Chairman, Department of Physiological Chemistry.

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TABLE I
The Clinical Diagnosis of Experimental Subjects

Subject, Age (yr.) and Sex	Clinical Diagnosis
J. M., F	Idiopathic hyperlipemia
H. P., 41, M	Idiopathic hyperlipemia
F. J., 38, M	Idiopathic hyperlipemia
H. A., 37, M	Idiopathic hyperlipemia, xanthoma tuberosum
L. S., 46, F	Idiopathic hyperlipemia, xanthoma tuberosum
L. R., 53, F	Diabetes mellitus, xanthoma tuberosum, angina
T. M., 34, F	Diabetes mellitus, xanthoma tuberosum, angina
R. K., 41, F	Diabetes mellitus, xanthoma tuberosum
W. S., 44, M	Diabetes mellitus, diabetic acidosis
H. B., 52, M	Diabetes mellitus, Kimmelstiel-Wilson Syndrome
F. F., 49, M	Diabetes mellitus, arteriosclerosis
G. E., 31, M	Diabetes mellitus
E. W., 38, F	Diabetes mellitus
R. W., 49, F	Diabetes mellitus
C. D., 50, M	Diabetes mellitus, hypothyroid, arteriosclerosis
C. A., 50, M	Diabetes mellitus, xanthoma tuberosum

ceding study.[13] Results obtained from the fractionation of serum from normal subjects, described in the preceding study,[13] have been included in the present investigation for comparison with the hyperlipemic subjects.

RESULTS

Isolation and Analysis of Serum Lipoprotein Fractions

Lipoprotein fractions were isolated from hyperlipemic subjects and characterized by their cholesterol, phospholipid and fatty acid ester content. Cholesterol and phospholipid were elevated over a wide range in the serum of hyperlipemic subjects (Table II) and were similar to the elevated levels reported in other investigations of serum lipids in idiopathic hyperlipemia[1,2,4,5,14] and diabetes mellitus.[1,2,15] Cholesterol:phospholipid ratios for hyperlipemic serum varied over a wide range from 0.56 to 1.50 and cannot be used as an indication of the hyperlipemic state.[16,17] Ahrens and Kunkel[18] analyzed serums from nephrotic and biliary cirrhotic subjects and suggested that a relative abundance of phospholipid (i.e., a low cholesterol:phospholipid ratio) stabilized serum and prevented the lactescence commonly observed in hyperlipemia. This observation was not confirmed in the present investigation. Lactescence was observed in subjects with both low and high cholesterol:phospholipid ratios (H. P. and G. E.), while the serum of one subject with a high cholesterol:phospholipid ratio (L. S.) was essentially clear. Fatty acid esters were elevated in the serum of hyperlipemic subjects (Table III). Fatty acid ester:cholesterol ratios varied from 0.58 to 2.78. These observations confirm the many studies which have shown that triglycerides are markedly elevated in hyperlipemic states.[1,2,4,9,14,17] The triglyceride content of serum may be calculated with several assumptions from cholesterol, phospholipid and total fatty acid ester analyses.[19] Total fatty acid esters are a less specific estimate of triglyceride content; however, the serum fatty acid ester:cholesterol ratio for hyperlipemic subjects is always greater and in most subjects markedly greater than the fatty acid ester:cholesterol ratio for normal or hypercholesterolemic subjects.[13]

Cholesterol, phospholipid and fatty acid esters are elevated in the chylomicron fractions isolated from hyperlipemic subjects (Tables II and III). Cholesterol:phospholipid ratios vary from 0.81 to 1.93 and fatty acid ester:cholesterol ratios vary from 0.66 to 3.20 in this fraction. It is difficult to establish the homogeneity of the chylomicron fraction and demonstrate that it represents only chylomicrons of alimentary origin. In the present investigation, the chylomicron fraction was isolated from serum by centrifugation through 0.15 M sodium chloride at 9,300 × g for thirty minutes. This procedure, which removes particles visible in the dark-field microscope together with a small amount of contaminating serum,[13] is similar to other procedures where the chylomicron fraction was isolated by centrifugation at 9,500 × g for ten minutes.[20–22] However, the composition of the chylomicron fraction suggests that some very low density

TABLE II

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of Hyperlipemic Subjects

Subject	Serum			Chylomicron			S_f 10–400			S_f 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Thirty-One)†															
	204.0	233.0	0.88	4.7	5.6	0.88	21.4	28.7	0.76	139	114	1.23	38.1	79.4	0.49
	27.6	33.3	0.07	1.8	2.2	0.28	9.4	13.5	0.12	23.4	23.2	0.10	9.5	20.3	0.07
Subjects with Idiopathic Hyperlipemia															
J. M.	471	514	0.92	296.0	233.0	1.27	119	178	0.67	38.1	65.5	0.58	6.9	26.8	0.26
H. P.	1,045	1,800	0.58	333.0	413.0	0.81	653	963	0.68	100.0	245.0	0.41	25.5	120.0	0.21
F. J.	345	358	0.96	13.7	12.3	1.11	201	184	1.09	108.0	99.5	1.09	19.8	52.8	0.38
H. A.	228	340	0.67	20.1	20.0	1.01	139	165	0.84	38.9	68.0	0.57	12.7	54.5	0.23
H. A.	560	543	1.03	117.0	62.5	1.87	397	350	1.13	47.0	65.0	0.78	12.4	45.0	0.28
L. S.	810	613	1.32	41.7	22.5	1.85	648	434	1.49	80.4	80.0	1.01	21.6	55.6	0.39
Subjects with Idiopathic Hyperlipemia and Diabetes Mellitus															
L. R.	425	430	0.99	35.0	25.5	1.37	285	243	1.17	106.0	98.0	1.08	36.7	80.5	0.46
T. M.	793	1,405	0.56	85.4	99.0	0.86	628	1,118	0.56	46.3	72.5	0.64	9.8	43.0	0.23
R. K.	1,880	1,790	1.05	756.0	605.0	1.25	981	1,072	0.91	13.5	28.8	0.47	14.6	62.9	0.23
L. B.	312	358	0.87	13.9	16.3	0.85	156	168	0.93	70.9	79.4	0.89	38.8	82.5	0.47
F. F.	308	390	0.79	36.1	34.1	1.06	155	195	0.79	76.7	62.5	1.23	23.4	66.0	0.35
G. E.	800	533	1.50	494.0	256.0	1.93	216	166	1.30	15.6	21.9	0.71	18.4	58.1	0.32
E. W.	593	613	0.97	57.0	51.5	1.11	390	355	1.10	156.0	148.0	1.05	18.2	53.8	0.34
R. W.	470	593	0.79	13.5	13.4	1.01	315	412	0.76	115.0	90.0	1.28	31.6	62.5	0.51
C. D.	405	470	0.86	31.3	27.5	1.14	227	213	1.07	129.0	146.0	0.88	12.2	44.3	0.28
C. A.	667	754	0.88	139.0	117.0	1.19	443	508	0.87	22.5	40.6	0.55	16.3	69.1	0.24
Subjects with Diabetic Acidosis															
W. S.	578	795	0.73	87.7	69.0	1.27	479	615	0.78	35.2	55.0	0.64	9.4	40.5	0.23

* Cholesterol: phospholipid ratio.
† First row represents the mean and the second row the standard deviation.

lipoproteins are isolated together with alimentary chylomicrons from the serums of hyperlipemic subjects. Chylomicrons isolated from two normal subjects during alimentary lipemia* had fatty acid ester: cholesterol ratios greater than 3.0. Chylomicron fractions isolated from hyperlipemic subjects had, with one exception (H. P.), lower fatty acid ester: cholesterol ratios. Furthermore, these ratios tended to be only slightly greater than ratios for the corresponding S_f 10–400 lipoprotein fractions. While decreased fatty acid ester: cholesterol ratios in the chylomicron fraction might be explained by intravascular lipolysis during the delayed removal of chylomicrons from the blood, several investigators have suggested that chylomicrons are removed as particles.[22] The lowered fatty acid ester: cholesterol ratio might indicate that very low density lipoproteins related to the S_f 10–400 lipoprotein fraction are also isolated in the "chylomicron" fraction.

The S_f 10–400 lipoprotein fraction is elevated in all hyperlipemic subjects (Tables II and III). This elevation is the most significant alteration in the serum lipoprotein distribution. Cholesterol: phospholipid ratios vary from

* These chylomicron fractions were obtained four hours after the administration of 90 gm. Lipomul® Oral.

TABLE III

The Distribution of Fatty Acid Ester (FAE) in Serum and Serum Lipoprotein Fractions of Hyperlipemic Subjects

Subject	Serum		Chylomicron		S_f 10–400		S_f 3–9		High Density	
	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*	FAE (mEq. %)	Ratio*
Normal Subjects (Eleven)†										
	0.89	0.49	0.05	1.04	0.23	1.13	0.40	0.33	0.14	0.48
	0.22	0.15	0.05	1.03	0.14	0.22	0.08	0.08	0.03	0.12
Subjects with Idiopathic Hyperlipemia										
J. M.	9.20	1.97	6.45	2.18	2.03	1.71	0.43	1.13	0.12	1.74
H. P.	29.1	2.78	10.6	3.20	17.2	2.63	1.06	1.06	0.27	1.06
F. J.	1.99	0.58	0.09	0.66	1.30	0.65	0.33	0.31	0.13	0.66
H. A.	6.76	1.21	1.39	1.19	4.73	1.19	0.38	0.81	0.13	1.06
L. S.	5.97	0.74	0.40	0.96	4.97	0.77	0.48	0.60	0.16	0.74
Subjects with Idiopathic Hyperlipemia and Diabetes Mellitus										
T. M.	18.6	2.35	1.81	2.12	13.9	2.21	0.45	0.97	0.14	1.43
R. K.	26.5	1.41	11.1	1.47	13.1	1.34	0.16	1.19	0.16	1.10
G. E.	10.6	1.33	7.46	1.51	2.03	0.94	0.12	0.77	0.15	0.82
C. D.	3.78	0.93	0.41	1.31	2.15	0.95	0.72	0.56	0.12	0.92
Subjects with Diabetic Acidosis										
W. S.	9.58	1.66	1.51	1.72	7.32	1.53	0.44	1.25	0.13	1.38

* Fatty acid ester (mEq.) × 100:cholesterol (mg.) ratio.
† First row represents the mean and the second row the standard deviation.

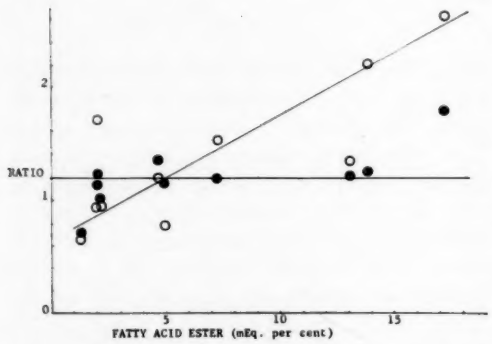

FIG. 1. The fatty acid ester:phospholipid ratios (●), and fatty acid ester:cholesterol ratios (○), plotted as a function of the fatty acid ester content of the S_f 10–400 lipoprotein fractions isolated from hyperlipemic subjects.

0.56 to 1.49 while fatty acid ester:cholesterol ratios vary from 0.65 to 2.63 (Tables II and III). Many of the individual ratios are markedly different from the ratios found for S_f 10–400 lipoproteins isolated from normal serum, and suggest that the composition of $S_f > 10$ lipoproteins is often modified in hyperlipemic subjects. Havel et al.[11] have reported elevated cholesterol:phospholipid ratios in the S_f 10–400 lipoproteins of three hyperlipemic subjects.

Lipoprotein increments in hyperlipemic subjects have been found specifically in the S_f 20–100, S_f 100–400 and S_f 100–40,000 lipoprotein fractions, and throughout the S_f 0–40,000 lipoprotein spectrum, by analytical ultracentrifugation.[7] Oncley et al.[23] have stud-

TABLE IV

The Distribution of Cholesterol and Phospholipid in S_f 10–400 Lipoprotein Subfractions of Hyperlipemic Subjects

Subject	Date	S_f 10–400			S_f 10–400 (D < 1.005)			S_f 10–400 (D > 1.005)		
		Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Five)†										
		27.8	35.6	0.78	15.2	22.5	0.68	9.8	10.6	0.92
		8.9	10.7	0.06	5.4	7.9	0.06	1.8	1.6	0.13
Subjects with Hyperlipemia										
L. S.	4/8/59	343	273	1.31	220	184	1.20	124.0	91.7	1.35
L. S.	4/27/59	408	316	1.29	297	229	1.30	82.3	61.0	1.35
R. K.	4/8/59	631	941	0.67	528	818	0.64	3.5	8.3	0.42
R. K.	4/29/59	981	1,072	0.91	875	1,005	0.87	8.1	19.0	0.43
R. K.	5/13/59	406	517	0.78	378	467	0.81	24.9	28.8	0.86
R. K.	6/15/59	202	349	0.58	185	292	0.62	8.6	14.6	0.59
T. M.	4/13/59	319	500	0.64	288	494	0.58	18.8	24.6	0.76
L. B.	5/5/59	156	168	0.93	108	139	0.77	26.4	26.3	1.00
F. F.	5/20/59	155	195	0.79	115	188	0.60	6.6	8.1	0.80

* Cholesterol:phospholipid ratio.

† First row represents the mean and the second row the standard deviation.

ied the S_f 10–400 lipoprotein distribution in normal subjects and reported three lipoprotein subfractions with increasing density, S_f 40–160, S_f 22–38 and S_f 7–13. These studies demonstrate that the S_f 10–400 lipoprotein fraction is composed of several subfractions varying in composition and physical properties. Variations in composition found in the present investigation may then represent increments in specific lipoprotein subfractions. The flotation rate, S_f, is a function of density and density is correlated with the triglyceride content of a lipoprotein molecule.[7,11,21,23–26] If the relative triglyceride content is increased, the relative cholesterol, phospholipid or protein content of this molecule must be decreased. Lipoprotein molecules with an increased relative triglyceride content are concentrated in the upper portion of the S_f 10–400 lipoprotein spectrum. When fatty acid ester:phospholipid and fatty acid ester:cholesterol ratios were plotted against the fatty acid ester content of the S_f 10–400 lipoprotein fraction, the fatty acid ester:phospholipid ratio was constant; however, the fatty acid ester:cholesterol ratio increased with increasing S_f 10–400 fatty acid esters (Fig. 1). These observations suggest (1) a constant triglyceride to phospholipid ratio is important in lipoprotein formation and stability and (2) the triglyceride and phospholipid are increased relative to cholesterol in the very low density lipoproteins.

S_f 10–400 lipoprotein fractions isolated from hyperlipemic subjects were separated into two subfractions, S_f 10–400 (D < 1.005) and S_f 10–400 (D > 1.005) by ultracentrifugation in 0.15 M sodium chloride.[13] S_f 10–400 lipoproteins with an elevated cholesterol:phospholipid ratio (1.30) contained a significant quantity of lipoprotein with a density greater than 1.005, while S_f 10–400 lipoproteins with lower cholesterol: phospholipid ratios (0.58 to 0.93) contained much smaller amounts of these lipoproteins (Table IV). These observations suggest that specific lipoprotein subfractions in the lower S_f range[13,21,23] are elevated in hyperlipemic subjects when the cholesterol:phospholipid ratio is elevated.

The concentration of S_f 3–9 lipoproteins

TABLE V

The Distribution of Cholesterol and Phospholipid in S_f 3–9 and Repurified S_f 3–9 (A) Lipoprotein Fractions of Hyperlipemic Subjects

Subject	Date	S_f 3–9			S_f 3–9 (A)		
		Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Eight)†							
		134 25.6	106 22.3	1.27 0.06	118 25.9	83.7 17.0	1.41 0.10
Subjects with Hyperlipemia							
L. S.	4/8/59	137	127	1.08	125	98.8	1.27
L. S.	4/27/59	103	93.1	1.11	82.5	71.0	1.16
R. K.	4/8/59	6.3	12.5	0.50	4.5	10.0	0.45
R. K.	4/29/59	13.5	28.8	0.47	8.6	14.7	0.59
R. K.	5/13/59	46.3	69.4	0.67	38.8	41.6	0.93
R. K.	6/15/59	40.0	50.0	0.80	27.5	25.3	1.09
T. M.	4/13/59	76.7	79.4	0.97	55.4	50.5	1.10
L. B.	5/5/59	70.9	79.4	0.89	61.7	56.6	1.09
F. F.	5/20/59	76.7	62.5	1.23	70.9	56.9	1.25

* Cholesterol:phospholipid ratio.
† First row represents the mean and the second row the standard deviation.

tends to be decreased in subjects with hyperlipemia (Tables II and III). This observation confirms other studies on iodopathic hyperlipemia and nephrosis in which lipoprotein concentrations were estimated by analytical ultracentrifugation,[7] homogeneous density[11] and density gradient[12] procedures. Analytical ultracentrifugation studies by several investigators have shown normal[27,28] and elevated[29] S_f 0–12 lipoproteins in subjects with hyperlipemia and diabetes mellitus, and decreased S_f 0–12 lipoproteins in subjects with diabetic acidosis.[30] In the present investigation, the S_f 3–9 lipoprotein fraction was decreased in six hyperlipemic subjects with associated diabetes mellitus and in one subject with diabetic acidosis; it was normal in only three hyperlipemic subjects with associated diabetes mellitus.

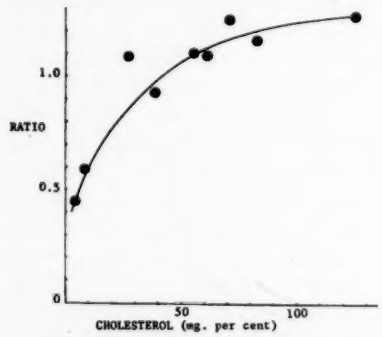

FIG. 2. The cholesterol:phospholipid ratios (●) plotted as a function of the cholesterol content of the repurified S_f 3–9 (A) lipoprotein fractions isolated from hyperlipemic subjects.

Cholesterol:phospholipid ratios, 0.47 to 1.28, are lower than normal, while fatty acid ester:cholesterol ratios, 0.31 to 1.25, are higher than normal for the S_f 3–9 lipoprotein fraction (Tables II and III). Since S_f 3–9 lipoproteins separated by the density gradient procedure are contaminated with high density lipoproteins,[13] the decreased cholesterol:phospholipid ratio might reflect contamination. However, abnormal cholesterol:phospholipid ratios were found in S_f 3–9 lipoproteins repurified

TABLE VI

The Distribution of Cholesterol and Phospholipid in the High Density Lipoproteins of Hyperlipemic Subjects

Subject	Date	High Density			High Density (A)			High Density (T)		
		Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Normal Subjects (Eight)†										
		41.5	94.0	0.44	10.2	18.7	0.56	51.7	113.0	0.46
		7.5	13.8	0.05	5.7	11.5	0.09	9.8	19.9	0.05
Subjects with Hyperlipemia										
L. S.	4/8/59	39.7	97.5	0.41	12.8	28.8	0.44	52.5	126.0	0.42
L. S.	4/27/59	48.4	115.0	0.42	10.2	21.5	0.47	58.6	137.0	0.43
R. K.	4/8/59	9.6	43.8	0.22	1.6	7.6	0.14	11.2	51.4	0.22
R. K.	4/29/59	14.6	62.9	0.23	3.1	11.3	0.27	17.7	74.2	0.24
R. K.	5/13/59	34.4	117.0	0.29	11.0	28.5	0.39	45.4	146.0	0.31
R. K.	6/15/59	37.1	104.0	0.36	12.4	22.7	0.55	49.5	127.0	0.39
T. M.	4/13/59	27.5	83.5	0.33	24.3	28.5	0.88	51.8	112.0	0.46
L. B.	5/5/59	38.8	82.5	0.47	9.9	22.7	0.44	48.7	105.0	0.46
F. F.	5/20/59	23.4	66.0	0.35	3.5	8.3	0.42	26.9	74.3	0.36

* Cholesterol:phospholipid ratio.
† First row represents the mean and the second row the standard deviation.

TABLE VII

The Distribution of Cholesterol, Phospholipid and Fatty Acid Ester in Serum and Lipoprotein Fraction of a Hyperlipemic Subject with Diabetic Acidosis (W. S.) During Insulin Therapy

Date	Serum			Chylomicron			Sf 10–400			Sf 3–9			High Density		
	Therapy: NPH Insulin 35 Units, Regular Insulin 10 Units														
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
5/13/58	798	1,150	0.69	240.0	225.0	1.07	560.0	798.0	0.70	17.8	56.6	0.32	8.6	45.5	0.19
5/15/58	578	795	0.73	87.7	69.0	1.27	479.0	615.0	0.78	35.2	55.0	0.64	9.4	40.5	0.23
5/16/58	595	700	0.85	57.7	39.0	1.48	480.0	510.0	0.94	45.9	61.5	0.75	11.4	35.5	0.32
5/19/58	483	645	0.75	35.8	26.0	1.38	381.0	450.0	0.85	40.1	60.0	0.67	11.8	43.0	0.27
6/11/58	253	295	0.86	5.0	4.0	1.25	92.6	112.0	0.83	127.0	105.0	1.21	17.1	48.0	0.36
7/17/58	231	285	0.81	8.7	10.5	0.83	66.7	92.3	0.72	122.0	103.0	1.18	16.3	44.5	0.37
	FAE (mEq. %)	Ratio†		FAE (mEq. %)	Ratio†		FAE (mEq. %)	Ratio†		FAE (mEq. %)	Ratio†		FAE (mEq. %)	Ratio†	
5/15/58	9.58	1.66		1.51	1.72		7.32	1.53		0.44	1.25		0.13	1.38	
5/10/58	7.62	1.28		0.76	1.32		5.60	1.17		0.40	0.87		0.11	0.96	
5/19/58	6.03	1.25		0.39	1.09		4.53	1.19		0.27	0.68		0.08	0.68	

* Cholesterol:phospholipid ratio.
† Fatty acid ester (mEq.) × 100:cholesterol (mg.) ratio.

TABLE VIII

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of a Hyperlipemic Subject with Diabetes Mellitus and Xanthoma Tuberosum (R. K.) During Insulin, Estrogen and Unsaturated Fatty Acid Therapy

Date	Serum			Chylomicron			S_f 10–400			S_f 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Therapy: NPH Insulin 40 Units. Diabetic Diet, 1,500 cal.															
7/2/58	650	840	0.77	53.8	68.3	0.79	518	581	0.89	46.7	65.0	0.72	15.3	72.3	0.21
7/16/58	345	463	0.75	26.9	31.5	0.85	162	213	0.76	131	123	1.07	27.1	72.8	0.37
7/24/58	340	383	0.89	12.7	26.5	0.48	127	150	0.85	149	135	1.10	25.1	67.3	0.37
9/3/58	348	525	0.66	18.8	34.8	0.54	217	320	0.68	88.9	83.0	1.07	26.9	68.5	0.39
10/22/58	307	481	0.64	24.2	40.0	0.61	187	276	0.68	72.2	76.0	0.95	23.9	72.8	0.33
12/29/58	399	561	0.71	17.2	25.5	0.67	261	355	0.79	68.0	76.0	0.89	30.0	92.3	0.32
Additional Therapy: Manvene† 20 mg.															
2/17/59	585	718	0.82	51.2	50.5	1.02	448	531	0.84	48.7	62.5	0.78	22.0	67.8	0.32
4/8/59	1230	1610	0.76	559	606	0.92	631	941	0.67	6.3	12.5	0.50	9.6	43.8	0.22
Additional Therapy: Dietary Lipid Restricted to 50 gm. Lipomul® Oral and 20 gm. Animal Fat															
4/29/59	1880	1793	1.05	756	605	1.25	981	1072	0.91	13.5	28.8	0.47	14.6	62.9	0.23
5/13/59	535	748	0.72	20.6	27.5	0.75	406	517	0.78	46.3	69.4	0.67	34.4	117	0.29
6/15/59	313	525	0.60	29.1	55.6	0.52	202	349	0.58	40.0	50.0	0.80	37.1	104	0.36
8/20/59	395	515	0.77	26.3	36.0	0.73	250	265	0.94	62.5	79.5	0.79	31.5	92.3	0.34

* Cholesterol:phospholipid ratio.
† Manvene: 3-methoxy-16α-methyl-1,3,5(10)-estratriene-16β,17β-diol.

by centrifugation at density 1.063 (Table v). The cholesterol:phospholipid ratio for this lipoprotein fraction, S_f 3–9 (A), was proportional to the cholesterol content of this fraction (Fig. 2). Furthermore, low cholesterol:phospholipid ratios were found even in hyperlipemic subjects (H. P., E. W. and C. D.) with appreciable lipoprotein in the S_f 3–9 fraction (Table II). In this respect, the S_f 3–9 lipoproteins isolated from hyperlipemic subjects are similar to the major lipoprotein fraction, density 1.036 to 1.046, isolated from a subject with biliary cirrhosis.[31] It is interesting that phospholipid and triglyceride are both elevated with respect to cholesterol in this lipoprotein fraction.[31]

The concentration of high density lipoproteins tends to be decreased in the serum of hyperlipemic subjects (Table II). The total high density lipoprotein fraction obtained after repurification of the S_f 3–9 lipoproteins, High Density (T), is often found in a lower concentration (Table VI). This decrease in high density lipoproteins is correlated with a markedly elevated chylomicron fraction. When hyperlipemia is corrected, the high density lipoproteins increase in concentration (see R. K. Tables IV and VI; also Tables VII, VIII and IX).

The cholesterol:phospholipid ratio is decreased while the fatty acid ester:cholesterol ratio is elevated in the high density lipoproteins isolated from hyperlipemic subjects (Tables II, III and VI). These ratios may indicate the presence of atypical high density lipoproteins. However, a phospholipid fraction containing little or no cholesterol has been isolated from serum and characterized by several investigators.[11,32–34] The phospholipid in this fraction would contribute to the decreased cholesterol:phospholipid and elevated fatty acid ester:cholesterol ratios found in high density lipoproteins isolated from hyperlipemic subjects.

TABLE IX

The Distribution of Cholesterol and Phospholipid in Serum and Serum Lipoprotein Fractions of a Hyperlipemic Subject with Diabetes Mellitus and Xanthoma Tuberosum (C. A.) During Insulin and Unsaturated Fatty Acid Therapy

Date	Serum			Chylomicron			S_f 10–400			S_f 3–9			High Density		
	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*	Cholesterol (mg. %)	Phospholipid (mg. %)	Ratio*
Therapy: NPH Insulin 68 units.† Diabetic Diet 1,800 cal. Dietary Lipid Restricted to 80 gm. Lipomul Oral and 15 gm. Animal Fat															
1/7/60	667	754	0.88	139.0	117.0	1.19	443.0	508	0.87	22.5	40.6	0.55	16.3	69.1	0.24
1/11/60‡	545	568	0.96	59.4	51.3	1.16	381.0	351	1.09	46.7	53.8	0.87	30.8	84.1	0.37
1/12/60	508	433	1.17	19.1	22.2	0.86	371.0	323	1.15	45.0	52.5	0.86	27.9	78.4	0.36
1/15/60	465	481	0.97	54.4	42.5	1.28	279.0	300	0.93	77.5	75.0	1.03	26.6	68.8	0.39
2/1/60	268	306	0.88	10.9	13.8	0.79	92.5	146	0.63	90.0	70.0	1.29	30.4	69.7	0.44

* Cholesterol: phospholipid ratio.
† Insulin requirement decreased to 30 units during therapy.
‡ One unit of Lipomul administered intravenously.

The Composition and Distribution of Lipoprotein Fractions in the Serum of Hyperlipemic Subjects During Therapy

The composition and distribution of serum lipoproteins were studied during the administration of insulin to a subject in diabetic acidosis, and during the administration of insulin and unsaturated fatty acids as Lipomul® Oral to two subjects with hyperlipemia and associated diabetes mellitus.*

Serum Lipoproteins in Diabetic Acidosis and During Insulin Therapy. A forty-four year old white man (W. S.) was admitted to the University Hospital with diabetic acidosis. Blood specimens were obtained for serum lipid and lipoprotein studies on admission and during insulin therapy. The initial lipoprotein distribution, elevated chylomicron and S_f 10–400 lipoprotein fractions together with decreased S_f 3–9 and high density lipoprotein fractions, demonstrated pronounced hyperlipemia (Table VII). With insulin therapy, the serum concentrations of cholesterol, phospholipid and fatty acid esters decreased rapidly, were essentially normal at one month, and were maintained at this level for a second month. The chylomicron fraction demonstrated a marked initial decrease but did not revert immediately to normal. This fraction was normal at one month. Although a marked decrease in S_f 10–400 lipoproteins also occurred, they were still somewhat elevated after two months. The concentration of S_f 3–9 lipoproteins increased during therapy and was maintained at a normal level one month after therapy was instituted. The cholesterol: phospholipid ratio of the S_f 3–9 lipoprotein fraction increased to the normal range while the fatty acid ester: cholesterol ratio for this fraction decreased toward the normal range. The concentration of high density lipoproteins increased but did not reach a normal level in the observation period. The cholesterol: phospholipid ratio for the high density lipoprotein fraction increased together with the increase in concentration.

These observations are somewhat different from those of two investigations of the lipoprotein distributions in diabetic acidosis employing analytical ultracentrifugation. Tuller et al.[27] showed that elevated S_f 12–400 lipoproteins were lowered rapidly with the administration of insulin to subjects in diabetic acidosis, but found little or no alteration in the S_f 0–12 lipoproteins before and during therapy. On the other hand, Kolb et al.[30] observed that the S_f 0–12 lipoproteins were decreased in

* Serum lipids[2] and lipoprotein distributions[33] are normal in the majority of subjects with controlled diabetes mellitus. Hyperlipemia in controlled diabetic subjects probably represents idiopathic hyperlipemia augmented by diabetes mellitus.

diabetic acidosis; however, they reported that the S_f 0–12 lipoproteins increased during therapy to twice the normal serum concentration for this fraction.

Serum Lipoproteins in Hyperlipemic Subjects with Associated Diabetes Mellitus During Insulin, Estrogen, and Unsaturated Fatty Acid Therapy. A hyperlipemic subject with diabetes mellitus and xanthoma tuberosum (R. K., a forty-one year old white woman) was admitted to the University Hospital for the evaluation of diabetic control. Blood specimens were obtained on admission and during therapy. Insulin therapy was effective initially in controlling hyperlipemia (Table VIII). The chylomicron fraction decreased to normal. S_f 10–400 lipoproteins decreased markedly but not to normal. The S_f 3–9 and high density lipoproteins increased to normal. Cholesterol: phospholipid ratios in these fractions increased toward normal. However, hyperlipemia was not controlled with continued insulin therapy. Lipoprotein distributions obtained two, three and five months after insulin therapy was instituted showed that the S_f 10–400 lipoproteins were increasing and the S_f 3–9 lipoproteins decreasing. An estrogen derivative with low estrogenic potency, Manvene,®[36] had no effect on the hyperlipemia (Table VIII). The concentration of serum lipids continued to increase. The chylomicron and S_f 10–400 lipoprotein fractions became grossly elevated while the S_f 3–9 and high density lipoprotein fractions decreased to very low levels. The administration of Manvene was discontinued and the subject placed on a diet high in linoleic acid (Lipomul Oral). This diet controlled the hyperlipemia and partially reversed the abnormal lipoprotein distribution (Table VIII). The chylomicron fraction decreased. S_f 10–400 lipoproteins decreased markedly but remained elevated throughout the period of observation. S_f 3–9 and high density lipoproteins both increased.

The control of hyperlipemia by linoleate administration was confirmed in a second study. A hyperlipemic subject with associated diabetes mellitus and xanthoma tuberosum (C. A., a fifty year old white man), was admitted to the University Hospital for the evaluation and control of hyperlipemia and xanthoma. Diabetes mellitus was controlled with the administration of NPH insulin, 68 units. However, hyperlipemia persisted during insulin therapy. A low fat diet had not been successful in reducing hyperlipemia. The subject was placed on a diet high in linoleate (Lipomul Oral). Serum lipid analyses and lipoprotein distribution studies showed that this diet controlled the hyperlipemia (Table IX). The chylomicron and S_f 10–400 lipoprotein fractions were markedly decreased and the S_f 3–9 and high density lipoprotein fractions increased. Cholesterol: phospholipid ratios in the latter fractions increased to the normal range. The insulin requirement of the subject decreased from 68 units to 30 units during unsaturated fatty acid therapy. These observations confirm the study of Kinsell et al.[37] who showed that hyperlipemia in diabetic subjects is controlled by the oral administration of linoleic acid.

COMMENTS

The lipoprotein pattern found in the serum of hyperlipemic subjects is characterized by elevated chylomicron and S_f 10–400 fractions and diminished S_f 3–9 and high density lipoprotein fractions. Variations within this pattern occur: gross increments in the chylomicron fraction, changes in composition and S_f distribution within the S_f 10–400 fraction, variations in the composition of the S_f 3–9 fraction. The hyperlipemia of diabetic acidosis is rapidly corrected by adequate insulin therapy. Dietary substitution of fats high in linoleic acid content greatly improves the lipoprotein pattern in idiopathic hyperlipemia.

The lipoprotein distribution in hyperlipemia and the factors involved in the pathogenesis and rational therapy of this condition can be interpreted on the basis of our current concept of the origin and metabolic interrelationships of serum lipoproteins. Kruger et al.[38] recently proposed a scheme outlining the path of lipid transport. Alimentary lipids initially enter the blood as chylomicrons via the lymphatics. The chylomicrons are metabolized extravascularly, presumably in the reticuloendothelial system,[39–41] liberating free fatty acids which

are transported in the plasma bound to albumin[22] and high density lipoproteins which also return to the plasma. The free fatty acids form a common pool with those elaborated by adipose tissue. The S_f 3–9 and S_f 10–400 lipoproteins constitute a system for lipid transport independent of the chylomicron-high density lipoprotein system. S_f 3–9 and S_f 10–400 lipoproteins are synthesized in the liver.[42,43] The fatty acid ester components of these lipoproteins are synthesized in the liver from the free fatty acids pool. The relative output of these two lipoproteins is governed by the availability of free fatty acids; high levels of free fatty acids leading to increased production of S_f 10–400 molecules at the expense of S_f 3–9 molecules. The S_f 10–400 lipoproteins are subsequently converted to S_f 3–9 lipoproteins,[12] this process also presumably occurring in the reticuloendothelial system, with the liberation of free fatty acids to the vascular pool.

Two mechanisms for the production of hyperlipemia immediately suggest themselves: either an overproduction of S_f 10–400 lipoproteins in the presence of a large free fatty acids pool, or a lipolytic or "clearing" defect in the conversion of S_f 10–400 lipoproteins to S_f 3–9 lipoproteins. In the case of S_f 10–400 underutilization it is likely that there would be a concommitant underutilization of chylomicrons since the site and mechanism of their degradation appear to be similar if not identical. In a recent review, Olson[3] has discussed overproduction and underutilization as factors in the genesis of hyperlipemias. He presented a scheme of lipid transport similar in some respects to that just outlined, but with one important difference: in his scheme chylomicrons can arise from adipose tissue. Recent studies by Savage[44] et al. indicate that fatty acids are not mobilized from the depots as triglycerides.

The term "chylomicron" has led to considerable confusion in the literature. Some authors have attributed all lactescence in serum to chylomicrons. However, very low density lipoproteins of hepatic origin, S_f 10–400, may contribute considerable lactescence to serum. In this paper the term "chylomicron" is used in two distinct senses. (1) they are operationally defined by the method used for their isolation and (2) they are functionally defined according to their origin and fate in the scheme outlined. There is little difficulty equating these two definitions as applied to chylomicrons found in serums from normal subjects under normal conditions. The S_f spectrum of the low density lipoproteins of hepatic origin is such that they are readily separated from "true" or alimentary chylomicrons by the technics used in the present investigation. However, in the hyperlipemic states the S_f spectrum of the low density lipoproteins may be so distorted as to overlap with that of the alimentary chylomicrons and thus preclude adequate separation by available methods. In the present study chemical analysis of the chylomicron and S_f 10–400 lipoprotein fractions indicates that the "chylomicron" fraction isolated from the serums of hyperlipemic subjects contains very low density lipoproteins rich in triglyceride with a flotation rate similar to alimentary chylomicrons, but differing from them in composition. The relative contribution of alimentary chylomicrons to the "chylomicron" fraction cannot be estimated until specific methods for their differentiation are available.

An increase in the S_f 10–400 lipoprotenis, whether resulting from underutilization or overproduction, is accompanied by a decrease in the S_f 3–9 lipoproteins. Underutilization, or decreased conversion of S_f 10–400 to S_f 3–9 lipoprotein, would be expected to contribute to a diminished S_f 3–9 fraction. Several experimental results support the role of decreased S_f 10–400 catabolism in the genesis of hyperlipemia. The S_f 10–400 lipoproteins labeled in their protein moieties with I^{131} are converted more slowly to labeled S_f 3–9 lipoproteins in hyperlipemic subjects with nephrosis than in normal subjects.[12] Orally administered I^{131}-labeled triolein remains as triglyceride in the chylomicron and S_f 10–400 fractions of hyperlipemic subjects for much longer periods of time than in normal subjects.[38] These differences, however, can also be at least partially explained by the increased dilution in the enlarged chylomicron and S_f 10–400 pools, and do not necessarily reflect a diminished rate of

catabolism. Underutilization has been established in certain instances of familial hyperlipemia by Havel and Gordon.[45] These investigators demonstrated the absence of clearing factor in the serums of three hyperlipemic siblings in response to the administration of heparin. Klein et al.[46] have recently reported that hyperlipemic subjects as a group elaborate less clearing factor than normal subjects in response to equivalent doses of heparin.

Overproduction of S_f 10–400 lipoprotein in the presence of a large free fatty acids pool would be expected to lead to diminished synthesis of S_f 3–9 lipoprotein on the basis of the proposed scheme. The hyperlipemia seen in diabetic acidosis can be readily explained by the marked elevation of free fatty acids found in this condition.[47] The effect of carbohydrate metabolism on lipid transport has recently been investigated. Havel[48] has shown that the ingestion of large amounts of glucose by normal subjects causes the level of very low density lipoproteins ($S_f > 10$) to fall significantly below fasting levels. Albrink et al.[49] have demonstrated a marked reduction in alimentary lipemia when glucose was administered together with fat as compared with the ingestion of fat alone. This effect was interpreted as resulting from the known free fatty acids lowering effect of glucose on free fatty acids.[50,51] Glucose appears to both inhibit the release of fatty acids from adipose tissue and also to stimulate fatty acid uptake by this tissue. It seems reasonable to expect that removal of free fatty acids from the albumin in the plasma would facilitate the lipolysis of triglyceride in the chylomicrons and S_f 10–400 lipoproteins since albumin is known to act as an acceptor of free fatty acids in the clearing reaction. (It might be noted here that the hypoalbuminemia in nephrosis has been implicated[3] as a factor in the production of hyperlipemia in this disease.) Therefore elevated free fatty acids would appear to lead to hyperlipemia by causing an increase in the synthesis of S_f 10–400 lipoproteins and by slowing the transformation of chylomicrons and S_f 10–400 lipoproteins by interfering with lipolysis.

The efficacy of dietary linoleate in lowering the blood lipids in hyperlipemia has been amply demonstrated by Kinsell and co-workers.[37] We have confirmed this effect in two patients with idiopathic hyperlipemia and associated diabetes mellitus. The mechanism of this effect is still obscure. Hellman and Rosenfeld[52] have recently demonstrated that a high corn oil diet results in a marked diminution in plasma cholesterol in a hyperlipemic subject which can be accounted for quantitatively by a corresponding increase in fecal sterols. The bile acid excretion remained unaltered. It is not known at present whether unsaturated fatty acids are effective by correcting the primary lesion in hyperlipemia or whether they enhance compensatory mechanisms which control blood lipid levels.

SUMMARY

Hyperlipemia is characterized by elevated chylomicron and S_f 10–400 and lowered S_f 3–9 and high density lipoprotein fractions. Chemical analyses suggest that the chylomicron fraction contains very low density lipoproteins of hepatic origin as well as chylomicrons of alimentary origin.

The composition and distribution of lipoprotein molecules within S_f 10–400 subfractions ($D < 1.005$ and $D > 1.005$) suggests that S_f 10–400 lipoprotein subfractions are not elevated proportionally in all hyperlipemic subjects. Chemical analyses indicate that a constant triglyceride:phospholipid ratio is important in the formation and stability of S_f 10–400 lipoproteins.

Atypical lipoproteins with low cholesterol:phospholipid and high fatty acid ester:cholesterol ratios are found in the S_f 3–9 lipoprotein fractions of hyperlipemic subjects.

Hyperlipemia is corrected by the administration of insulin to a subject with diabetic acidosis and the administration of linoleate to two subjects with idiopathic hyperlipemia and associated diabetes mellitus. The sequences in serum lipoprotein metabolism in hyperlipemic states are discussed with reference to overproduction and underutilization. The chylomicron fraction is defined operationally and functionally and the relative contribution of alimentary chylomicrons and endogenous lipoproteins to hyperlipemia discussed.

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Inborn Errors of Sphingolipid Metabolism

ALFRED G. KNUDSON, JR., M.D., PH.D.*

IT is my intent to gather together facts pertaining to the lipidoses which might serve to focus on current problems and to call attention to possible therapeutic approaches. The particular lipidoses to be discussed involve the accumulation in tissues of sphingolipids and may be referred to as sphingolipidoses. They may be classified as "inborn errors of sphingolipid metabolism" on the basis that they are determined by single factor inheritance and that sphingolipid metabolism is apparently not normal. From what we know of inborn errors it is a reasonable hypothesis that a single basic enzymatic defect is present in each disorder. The identification of these might stimulate a more rational approach to therapy, which is pitifully inadequate for these conditions at present. Four principal conditions will be considered: Gaucher's disease, Niemann-Pick disease, amaurotic family idiocy and familial leucodystrophy.

GAUCHER'S DISEASE

In its classic form, as described originally in 1882 by Gaucher[1] and other early workers, Gaucher's disease is a chronic disorder characterized by marked splenomegaly, hepatomegaly, pigmentation of the skin, hemorrhagic tendency and susceptibility to infection. By the time of the review by Hoffman and Makler[2] it was apparent that the disease could occur at virtually any age; almost half of the cases reported were in children. Death from hemorrhage, and possibly also from infection, are thought to be due to secondary hypersplenism and may be averted to a great extent by splenectomy. Patients with chronic Gaucher's disease are subject to disability from bone destruction as emphasized by Pick.[3] Severe pulmonary involvement has been reported by Myers[4] and others. Another localized involvement, namely, of brain, is still more severe as pointed out so strikingly by the report of Oberling and Woringer.[5] This form of the disease is characterized by death in infancy with decerebrate rigidity. The clinical picture of Gaucher's disease has been complicated still further by a few reports, such as that by Reiss and Kato,[6] of the delayed onset of severe and fatal brain involvement. Finally, as emphasized by Reich et al.,[7] the adult form of the disease may be relatively benign.

Broadly speaking three main forms of Gaucher's disease may be recognized: Type 1, noncerebral; Type 2, cerebral, acute; Type 3, cerebral, chronic. The question naturally arises whether these are all variants of the same disease or actually different diseases.

It has been clear since the early reports of Bovaird[8] and Collier[9] that Gaucher's disease is often familial. Hoffman and Makler[2] noted a familial incidence in approximately one-third of the affected families. There are some reports that more than one generation may be involved in one family.[10] There is considerable disagreement on the mode of inheritance. Groen,[11] for example, believes that the condition is dominantly inherited. Herndon and Bender[12] suggest autosomal recessive inheritance. Hsia et al.[10] present evidence for three genetic forms of Gaucher's disease: (1) dominant, chronic, (2) recessive, chronic and (3) recessive, cerebral.

In Gaucher's disease there is an accumulation in affected organs of large, pale-staining cells (Gaucher cells), which have an abundant, "wrinkled" cytoplasm. The storage cells are

From the Department of Pediatrics, City of Hope Medical Center, Duarte, California.

* Chairman, Department of Pediatrics.

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seldom found in the brain; the principal change in the brain is an atrophy of neurones in those patients with clinical evidence of brain involvement. Norman et al.[13] believe these differences may be related to the duration of the disease. Mandlebaum and Downey[14] were the first to view the disease as a metabolic problem and expressed the opinion on the basis of their chemical investigations that an intracellular accumulation of an unusual lipid "not of a pure lipoid" occurs. The lipid is faintly sudanophilic although it is extracted by lipid solvents. It gives a strong periodic acid-Schiff reaction. Lieb[15] and Epstein[16] classified the storage material as kerasin, a cerebroside. Klenk[17] and others demonstrated that the principal splenic lipid is a glucocerebroside rather than a galactocerebroside as found normally. Rosenberg and Chargaff[18] recently confirmed this finding. Ottenstein et al.[19] have found the simultaneous occurrence of glucocerebrosides and galactocerebrosides. Parke[20] and Woolf[21] have reported the isolation of lactose- and sucrose-containing cerebrosides. Uzman[22] has described the recovery of a polycerebroside. There is no good evidence for cerebroside accumulation in brain.[23]

NIEMANN-PICK DISEASE

The original descriptions of Niemann-Pick disease by Niemann[24] and other early observers provided a relatively uniform clinical picture. As demonstrated in the review of Baumann et al.[25] these patients had the onset of an enlarging abdomen due to massive hepatosplenomegaly in early infancy, followed by the progressive development of dementia, paralysis and blindness, the last often associated with macular abnormality of the type seen in Tay-Sachs disease. Death from inanition and pneumonia usually occurred between the ages of six and eighteen months. There have been reports since of patients surviving well past the age of two years. Crocker and Farber[26] reported on seven patients who died within the first two years of life, and five who died between the ages of three and six years. In addition they described an unusual form of the disease in four patients of French Catholic ancestry from Nova Scotia. These patients die in the second decade of life although brain involvement begins early in life. Finally, these authors reported on two children whose spleens showed the typical pathologic changes of Niemann-Pick disease but in whom there was no brain involvement. Three cases have been reported in adults, two by both Pfändler[27] and Dusendschon[28] and one by Terry et al.,[29] without brain disease but with death in adulthood and severe pulmonary involvement. Again, there seem to be three main clinical forms of Niemann-Pick disease: Type 1, cerebral, acute or subacute; Type 2, cerebral, chronic; Type 3, noncerebral.

Since the early report of Knox et al.[30] an increased familial incidence has been noted. Videbaek[31] reviewed the literature and found that in twelve of seventy-three sibships there was more than one case. Parental consanguinity was reported three times. Freudenberg[32] observed the disease in both of identical twins. Although no genetic analysis has been reported, somatic recessive inheritance is suggested.[33] About half of the reported cases have been in Jewish people, a fact which cannot be explained by consanguinity and which indicates an elevated gene frequency among Jews.

Pick[34] demonstrated clearly that virtually all organs, but especially spleen, liver, lymph nodes, bone marrow, lung and brain, show an accumulation of large, pale-staining cells (Niemann-Pick cells), whose cytoplasm is foamy and apparently contains lipid droplets. These droplets are sudanophilic but are not stained with periodic acid-Schiff's and Bial's reagents. Bloom and Kern[35] demonstrated that the lipids accumulating in these cells are phospholipids. Klenk's finding[36] that these are largely sphingomyelin has been confirmed. However, in some cases[26] sphingomyelin is not so disproportionately elevated. A more detailed qualitative and quantitative account of the accumulating lipids is obviously needed.

AMAUROTIC FAMILY IDIOCY

Amaurotic family idiocy is a term originally applied by Sachs[37] to a condition of infancy in which progressive spastic paralysis, dementia and blindness led to death, usually from pneumonia, at about two years of age. The first

case of this disease had been reported by Tay[38] who had described the ophthalmoscopic finding so characteristic of this infantile form of amaurotic family idiocy. "I found the optic discs apparently quite healthy, but in the region of the yellow spot in each eye there was a conspicuous, tolerably defined, large white patch, more or less circular in outline, and showing at its center a brownish red, fairly circular spot, contrasting strongly with the white patch surrounding it."

In his first report Sachs[39] referred to this change in the macula lutea as a "cherry red" spot. The report of Kingdon[40] served to bring together the various findings of Tay and Sachs. Subsequently there were reports of older children in whom progressive paralysis, dementia and blindness developed and who also had alteration of the macula but associated with pigmentation. Batten[41] described a seven year old girl in whom failing vision developed at the age of six years and who began to deteriorate mentally. Her older sister had a similar history and subsequently epileptiform seizures developed. At the macula there was a reddish black spot surrounded by a pale area. Mayou[42] described three children in one family with similar findings, all beginning about six years of age. Spielmeyer[43] reported on four children in a sibship of five with similar onset and symptoms and presented the first autopsy findings. Such cases were characterized as a juvenile form of amaurotic idiocy.[44] Jansky[45] and Bielschowsky[46] described late infantile cases and Kufs[47] an adult case, but these are very rare and may represent variants of the juvenile form. However, without doubt, two main types of amaurotic family idiocy may be recognized: Type 1, infantile (Tay-Sachs disease) and Type 2, juvenile (Batten-Mayou or Vogt-Spielmeyer disease).

Both forms have been studied genetically. Slome[48] reviewed 200 cases of Tay-Sachs disease reported in the literature and analyzed 135 genetically. Variance analysis demonstrated a close fit for autosomal recessive inheritance. Of 127 instances in which the ethnic background was stated 109 were Jewish and eighteen were non-Jewish. The parents were first cousins in approximately 15 per cent of the cases in Jews and in approximately 35 per cent of the cases in Gentiles. Goldschmidt et al.[49] studied the incidence of the disease among Jews in Israel and found twenty-nine cases among 85,500 births of Ashkenasic (Central and Eastern European) extraction and two cases among 67,700 births of other extraction. The gene frequency for the Ashkenasi was estimated at 0.0109, an estimate which agrees very closely with that of Kozinn et al.[50] for Jews in New York City where the gene frequency for non-Jews was estimated at 0.0015. Aronson et al.[51] have recently published a genetic survey of 144 cases with essentially similar findings. There are no data which enable one to discriminate among mutation rate, heterozygote selection, and genetic drift as the cause for the high gene frequency among Jews. Interestingly, the Jews are not affected by juvenile amaurotic family idiocy which, on the other hand, has an increased incidence among Swedes. In his monumental work on the Swedish cases Sjögren[52] concludes that autosomal recessive inheritance is operative.

In both diseases the brain at autopsy is found to be very firm. Microscopically, neurones in all parts of the brain are greatly distended with a material stored in the cytoplasm, this finding being less marked in the juvenile form. The ganglion cells of the retina also display storage although this is less pronounced in the juvenile cases in which the more striking finding is degeneration of the outer nuclear layers, rods and cones. The storage cells in both diseases are not sudanophilic but give positive reactions with periodic acid-Schiff's and Bial's reagents. Klenk's discovery[53] that the lipid accumulating in Tay-Sachs disease represented a new class of lipids, the gangliosides, has been amply confirmed. Diezel[54] concludes from his histochemical study that gangliosides are found in the juvenile form as well although Tingey[55] has not been able to recover increased amounts of gangliosides. In the juvenile form the storage material is difficult to extract, a finding attributed to protein binding. It is apparent that further studies are needed in the juvenile form. Even in the infantile form there has not been a complete accounting of the lipids. Rosenberg

and Chargaff[56] have reported an increase in a polymeric mucolipid and a decrease in monomeric cerebroside.

FAMILIAL LEUCODYSTROPHY

Familial leucodystrophy is a diagnosis applied to certain familial forms of diffuse cerebral sclerosis, a category of disease characterized clinically by progressive paralysis, dementia and blindness and pathologically by extensive demyelination within the brain, especially the cerebrum. Generally speaking, the nonhereditary forms (so-called Schilder's disease) show the histochemical changes typically found in multiple sclerosis and in Wallerian degeneration. Deposits of sudanophilic lipids (mostly cholesterol esters) are abundant. In Pelizaeus-Merzbacher disease, which exhibits sex-linked recessive inheritance, sudanophilic lipids are present, but there is a generalized reduction of all myelin lipids. There are two genetic conditions, however, in which sphingolipids accumulate abnormally in the brain. These are metachromatic leucodystrophy and Krabbe's disease.

Cases of metachromatic leucodystrophy were early separated from sudanophilic types by Scholz,[57] Greenfield[58] and others. Extensive demyelination is accompanied by collections of abnormal material, some of which stains metachromatically (various shades of red and brown) with basic aniline blue dyes (e.g., toluidine blue). However, it should be pointed out that some cases have sudanophilic lipids, too. Metachromatic leucodystrophy has been reported at all ages although it seems to occur most often in childhood. All of these cases are fatal after a variable course which is briefer in younger children than in older patients. The clinical and pathologic variations reported have suggested to some investigators that more than one disease is included in this category although v. Hirsch and Peiffer[59] indicate that the variations noted may be manifestations of a single disease. They stress that the additional finding of metachromatic material in ganglion cells suggests a generic relationship to the other lipidoses. Its familial occurrence suggests autosomal recessive inheritance. Clinical diagnosis is facilitated by the finding of metachromatically staining lipid material in urine sediment.[60,61] Austin[62] and Jatskewitz[63] have both presented evidence that the accumulating lipid is a sulfatide, probably cerebroside sulfate.*

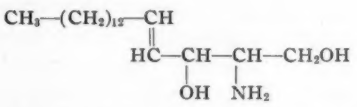
Krabbe's disease, so-called from the early report by Krabbe,[64] begins in infancy and runs a rapidly fatal course. Its familial incidence suggests autosomal recessive inheritance. Extensive demyelination is accompanied by axon degeneration and by marked cellularity. The cells present are epithelioid cells which accumulate in clusters, and so-called globoid cells which are large multinucleated cells. Histochemical studies demonstrate that the cytoplasmic material of both epithelioid and globoid cells is positive for periodic acid-Schiff's reagent. Blackwood and Cumings[65] and Diezel[66] believe that the substance is a cerebroside.

The familial leucodystrophies which involve sphingolipid metabolism are therefore tentatively classified as follows: Type 1, metachromatic leucodystrophy and Type 2, Krabbe's disease.

METABOLIC DEFECTS

On the basis of presently available information the diseases discussed here should be considered operationally as inborn errors of sphingolipid metabolism. It is essential, therefore, to review our knowledge of sphingolipid metabolism.

Sphingolipids have in common the basic compound sphingosine. The formula of this interesting compound is:



The predominant naturally occurring form is the *trans, erythro* isomer. In some sphingolipids sphingosine is replaced by its dihydro derivative. Sphingosine is synthesized enzymatically by a condensation and decarboxylation reaction involving palmityl-coenzyme A

* Marked elevation of cerebroside sulfate in cerebral white matter has recently been reported by Hagberg, B., Sourander, P., Svennerholm, L. and Voss, H. Late infantile metachromatic leucodystrophy of the genetic type. *Acta paediat.*, 49: 135. 1960.

and serine, the two terminal carbons of sphingosine being derived from the latter.[67]

The fatty acids that make the amide linkage with sphingosine (to form ceramides) are long chain, relatively saturated ones. Some fatty acids commonly present are noted as follows:

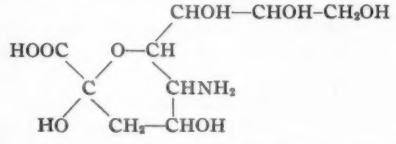
Stearic acid	$CH_3—(CH_2)_{16}—COOH$
Lignoceric acid	$CH_3—(CH_2)_{22}—COOH$
Cerebronic acid	$CH_3—(CH_2)_{21}—CH(OH)—COOH$
Nervonic acid	$CH_3—(CH_2)_7—CH=CH—(CH_2)_{13}—COOH$

It has been observed by Klenk[23] and others that stearic acid and cerebronic acid are found particularly in cerebral sphingolipids while lignoceric acid is found especially in splenic sphingolipids. It is probable that the acyl donors in ceramide syntheses are acyl-coenzyme A derivatives.

The sphingolipids may be categorized according to the groups linked to the terminal hydroxyl group of sphingosine:

sphingomyelin—phosphocholine
cerebrosides—hexose
"globosides"—hexose(s), hexosamine(s)
gangliosides—hexose(s), hexosamine(s), neuraminic acid

The hexose is generally galactose. Neuraminic acid, whose structure is shown,



is a very interesting nonulosaminic acid first described by Klenk[53] in gangliosides and by Blix[68] in mucosubstances. Its presence is detected by its chromogenic reaction with Bial's reagent.

Theoretically, the metabolic pathway from sphingosine to sphingolipids might proceed by addition first to the amino group or to the terminal hydroxyl group. In the case of cerebroside synthesis both possibilities have been demonstrated. Burton et al.[69] have shown that a ceramide may be an intermediate and Cleland and Kennedy[70] have shown that psychosine may be an intermediate. The galactose was donated in the prior instance by uridine diphosphate galactose. Sribney and Kennedy[71] have demonstrated that ceramide may react *in vitro* with cytidine diphosphate choline to give sphingomyelin. Curiously, however, only ceramides of *threo*-sphingosine were effective acceptors.

With respect to ganglioside synthesis there is a question whether the carbohydrate side chain is added in one step or in several steps. Although there is no answer presently available, Burton et al.[72] suggest the interesting possibility that the carbohydrate moiety may be added intact from a nucleotide. The group of sphingolipids reported by Klenk and Lauenstein[73] and called "globosides" by Yamakawa and Suzuki[74] present a slightly different question, i.e., whether they are formed by one or more reactions from ceramides or whether they are formed by the degradation of gangliosides.

Virtually nothing is known about sulfatide derivatives of glycolipids. Lipmann[75] has suggested that the formation of sulfate esters may be generalized as follows:

(1) inorganic sulfate + ATP ⇄ adenosine −5′-phosphosulfate (APS) + pyrophosphate
(2) APS + ATP → adenosine −3′-phosphate −5′-phosphosulfate (PAPS) + ADP
(3) PAPS + ROH (Acceptor) → PAP + ROS

Whether hydrolytic sulfatases are involved in the degradation of these compounds and whether such sulfatases are defective in leucodystrophy is purely speculative.

Little, too, is known about the degradation of sphingolipids. Although there has been mention of sphingomyelinase, cerebrosidase and gangliosidase, there is scanty evidence for such enzymatic activities. Yet it is in this area that many workers believe the enzyme defects occur in the inborn errors of sphingolipid metabolism. Considerable caution should be practiced here, however, until more details are known.

THERAPEUTIC APPROACHES

There is no adequate therapy at present for any of the conditions described. Hope comes from the belief that they are inborn errors of metabolism, and that inborn errors are, at least in some cases (e.g., galactosemia), treatable. Empirical measures are not to be scorned but a rational approach seems to demand a specification of the enzymatic defect in the various disorders in question.

Even when specific defects are known, it is very likely that new methods for dealing with them will be required. In galactosemia and phenylketonuria the defects can fortunately be circumvented because the accumulating substances are dependent upon dietary intake. In the conditions reviewed here dependence upon diet is more indirect. Obviously, other approaches to therapy should be considered.

There are two principal theoretic types of approach to the therapy of inborn errors of metabolism. One involves circumvention of the defect at the substrate level. Dietary regulation, as just mentioned, is one such approach; another is the administration of a metabolite with competitive or with feedback effects (e.g., cortisone in adrenal hyperplasia); and still another is the administration of antimetabolites which could competitively inhibit enzymatic reactions leading to the substance whose accumulation is undesirable. (It may be that extensive experience with cancer antimetabolites can be utilized in this area.)

The second type of approach is that which attempts to restore the defective enzyme. Two such approaches, both theoretic so far, may be suggested. The first involves correction of the enzyme defect, either by correcting a defect in the existing abnormal enzyme or by administering the normal enzyme; the second involves supplying the normal chemical mechanism for the synthesis of the enzyme, as by the administration of specific nucleic acid (either ribonucleic acid template or desoxyribonucleic acid genetic material) or by tissue or organ transplantation. Recent research in the areas of bacterial transformation and transduction, embryonic induction and acquired tolerance may prove to be applicable to this problem.

During the pursuit of the therapeutic goal much will be learned about the metabolism of lipids and, presumably, about their roles in cell physiology. This is a grossly deficient area of knowledge at present, much less being known about the cellular functions of these substances than about those of proteins and nucleic acids. That they have very important roles is suggested by studies on electron transport and electron insulation involving lipids. It may be hoped that the intellectual appeal of this aspect of the problem will attract the attention of the more academically inclined biochemists to these severe and thwarting clinical problems.

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Sphingomyelin Synthesis in Niemann-Pick Disease*

ALLEN C. CROCKER, M.D.* AND VIVIAN B. MAYS, B.S.†

LIPID LEVELS in the viscera of patients with Niemann-Pick disease are extraordinarily elevated. Although this increase involves the monoaminophosphatides and cholesterol as well, it is the surfeit of sphingomyelin which has consistently occupied the major attention of investigators. Because of these increased levels and also because of the enlargement of the organ, the spleen from such a patient may contain as much as 200 times the normal absolute amount of sphingomyelin. A list of possible mechanisms for this abnormality has been presented previously from this laboratory.[1] One possibility is that an acceleration of the rates of synthesis for the key lipids occurs in these patients and the studies herein were designed to explore this hypothesis.

For the purpose of the project, it was accepted that the residue of alkali-stable and acid-stable phosphatide from a washed lipid extract could be collected and taken to represent "sphingomyelin." It was further assumed that the radiophosphorus activity of this residue would be the result of biologic synthetic incorporation of the proffered inorganic P^{32} into sphingomyelin in a definite relation to native production of this lipid by the tissue under discussion. No attempts were made to investigate specific pathways or acceptance of labeled precursors other than the simple phosphate.

Few reports exist which give information on rates of synthesis of sphingomyelin relative to that of the total phospholipids. Hunter[2] stated that in twenty-four hours the specific activity of sphingomyelin in the livers of cats fed inorganic P^{32} was only about one-eighth that of the activity of lecithin. Hunter and Levy[3] reported that after subcutaneous injection the rate of incorporation of inorganic P^{32} into rat liver sphingomyelin was about 50 to 65 per cent that of the total phospholipids. Rates of incorporation into spleen were more nearly equal although at lower levels than the liver. Zilversmit et al.[4] showed in experiments with dogs that specific activities of sphingomyelin in liver ran considerably behind those of lecithin.

METHODS AND MATERIALS

The lipid analytic methods employed were the same as those previously described.[1] Portions of extract were dried on planchets for radioactivity counting (end window GM counter, with scaler). For the *in vitro* studies 1 mm. slices of tissue (average weight 100 to 150 mg.) were suspended in 25 ml. of borate-buffered (pH 7.4) Krebs-Ringer solution with added glucose, similar to the technic described by Popjak,[5] in a Dubnoff shaking incubator at 37°c. The human material was obtained at laparotomies being performed for the usual indications, with the special cooperation of the surgical and pathology departments to aid in the promptness of incubation.

Patient S. S., with Niemann-Pick disease, was presented as "Patient 18" in the earlier review.[1] Her spleen was found to contain 6.32 per cent of the fresh weight as sphingo-

From The Children's Cancer Research Foundation, The Children's Medical Center and the Department of Pathology, The Children's Hospital and Harvard Medical School, Boston, Massachusetts.

* Research Associate; † Laboratory Assistant.

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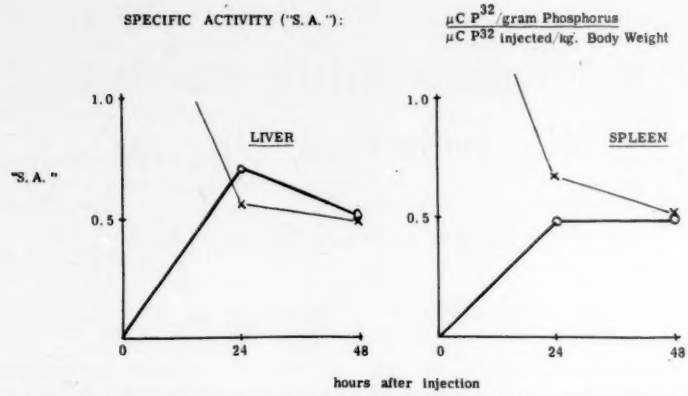

FIG. 1. *In vivo* demonstration of phospholipid synthesis. Intraperitoneal injection of inorganic P^{32} (2 to 4 μc.) in twelve normal adult rats that were sacrificed at twenty-four and forty-eight hours. Specific activity of inorganic and lipid phosphorus expressed in terms of dosage of isotope. O—O = lipid P^{32}; X—X = inorganic P^{32}.

myelin. Patient J. K., a forty month old child, had a very similar clinical picture with hepatosplenomegaly, pulmonary changes, mildly lipemic serum and normal intellectual development (the spleen yielded 6.29 per cent sphingomyelin). Patient P. M. is the younger sibling of Patient 11 in the previous report, and showed severe neurologic symptoms. Patient A. B. had mild organ enlargement and a lighter tissue abnormality (spleen 1.33 per cent sphingomyelin).

RESULTS

The *in vivo* experiments with normal adult rats (Fig. 1) indicated that the labeling of liver phospholipid reaches a maximum at or before twenty-four hours after injection of the tracer while the spleen shows a slower rate and lower general level. An average of 2.9 per cent of the injected isotope is found in the lipid phosphorus of the whole liver at twenty-four hours, falling to 2.3 per cent by forty-eight hours; in the whole spleen these figures are 0.078 per cent at twenty-four hours and 0.093 per cent at forty-eight hours. The specific activity of the sphingomyelin phosphorus reached in these tissues (Fig. 2) approaches that of the total lipid phosphorus, although sphingomyelin appears to be slower to reach maximal utilization of the inorganic P^{32}.

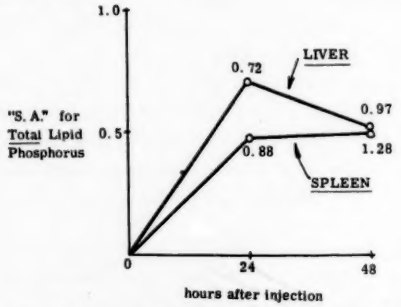

FIG. 2. Same experiments as shown in Figure 1, listing comparative data for sphingomyelin phosphorus. Numbers show ratio of sphingomyelin phosphorus specific activity to the specific activity of total lipid phosphorus at that point.

The *in vitro* (tissue slice) studies (Tables I and II) again showed that the hepatic activity in total phospholipid synthesis is greater than that of the spleen. With the animal work only about five minutes passed between removal of the specimen from the body and its establishment in the metabolic shaking incubator. In the human material this interval was usually several times as long which may account for lower absolute levels of specific activity. Sphingomyelin appears to be synthesized at relatively poorer rates by tissue slices, perhaps reflecting the unnatural circumstances or the short incubation time (Table III).

When specific activity is expressed in terms of grams of lipid phosphorus (as in the *in vivo*

TABLE I

Measurement of *In Vitro* Phospholipid Synthesis—Liver Slices*

Material Studied	Phospholipid Content (% of fresh wt.)	Specific Activity	
		mμc. lipid P^{32} / gram tissue	μc. lipid P^{32} / gram lipid P
Rats (2 animals)	2.00–2.17	16.8–17.2	19.8–21.0
Average		*17.0*	*20.4*
Human subjects			
General:			
M. T. (F, 1 yr., conv. hepatitis)	1.93	6.5	7.4
R. C. (M, 5 mo., conv. hepatitis)	1.68	4.6	7.0
K. S. (M, 6 yr., Gaucher's dis.)	2.77	8.5	9.5
Average		*6.5*	*8.0*
Niemann-Pick disease:			
J. K. (M, 3 yr.)	9.65	3.6	1.5
S. S. (F, 11 yr.)	14.6	20.0	4.1

* Incubated in borate-buffered Krebs-Ringer solution with 4 μC of inorganic P^{32} added; four hour data.

TABLE II

Measurement of *In Vitro* Phospholipid Synthesis—Spleen Slices*

Material Studied	Phospholipid Content (% of fresh wt.)	Specific Activity	
		mμc. lipid P^{32} / gram tissue	μc. lipid P^{32} / gram lipid P
Rats (4 animals)	1.18–1.36	1.5–9.1	3.6–19.2
Average		*6.8*	*13.9*
Human subjects			
General:			
M. T. (F, 1 yr., conv. hepatitis)	1.72	2.7	3.9
E. D. (F, 11 yr., spherocytosis)	1.25	4.9	8.2
C. W. (M, 10 yr., I.T.P.)	1.34	6.6	11.4
S. R. (F, 4 yr., Gaucher's dis.)	1.91	7.0	11.1
C. R. (F, 6 yr., Gaucher's dis.)	1.87	3.4	6.0
K. S. (M, 6 yr., Gaucher's dis.)	2.03	5.5	7.7
Average		*5.0*	*8.7*
Niemann-Pick disease:			
J. K. (M, 3 yr.)	9.50	3.8	1.4
S. S. (F, 11 yr.)	8.13	20.6	5.5

* Incubated in borate-buffered Krebs-Ringer solution with 4 μC of inorganic P^{32} added; four hour data.

work), the liver showed levels for sphingomyelin about two thirds those of the total phospholipids and the spleen about one half. If expressed per unit of tissue weight, the ratio is decreased in proportion to the tissue level of sphingomyelin; and both liver and spleen then show about 10 per cent as much sphingomyelin activity as total phospholipid.

When handling the material from the patients with Niemann-Pick disease, a critical problem arises regarding selection of units for specific activity because of the great residuum

TABLE III

Relative Sphingomyelin Specific Activity* $\left(\frac{\text{Sphingomyelin } P^{32}}{\text{Total Lipid } P^{32}}\right)$

Based on:	mμc. lipid P^{32}/ gram tissue	μc. lipid P^{32}/ gram lipid P
I. Liver slices		
Rat XIV	0.12	1.14
Rat XVI	0.01	0.09
R. C. (M, 5 mo., conv. hepatitis)	0.19	0.79
K. S. (M, 6 yr., Gaucher's dis.)	0.10	0.70
Average	*0.10*	*0.68*
Niemann-Pick disease:		
J. K. (M, 3 yr.)	0.04	0.07
S. S. (F, 11 yr.)	0.07	0.12
Average	*0.06*	*0.10*
II. Spleen slices		
Rat XIV	0.17	0.55
Rat XVI	0.13	0.43
E. D. (F, 11 yr., spherocytosis)	0.14	0.50
C. W. (M, 10 yr., I.T.P.)	0.06	0.23
S. R. (F, 4 yr., Gaucher's dis.)	0.13	0.47
C. R. (F, 6 yr., Gaucher's dis.)	0.16	0.58
K. S. (M, 6 yr., Gaucher's dis.)	0.11	0.46
Average	*0.13*	*0.46*
Niemann-Pick disease:		
J. K. (M, 3 yr.)	0.07	0.09
S. S. (F, 11 yr.)	0.07	0.10
Average	*0.07*	*0.10*
III. White blood cell suspensions		
B. (normal)	0.29	0.56
Niemann-Pick disease:		
P. M. (F, 2 yr.)	0.14	0.29
A. B. (M, 7 yr.)	0.13	0.37

* Sphingomyelin *in vitro* synthesis data, from the same experiments as presented in Tables I and II. Results given as ratio of sphingomyelin phosphorus radioactivity to that of total lipid phosphorus.

of lipid in these tissues. Utilization of radioactivity per gram of tissue rather than per gram of lipid phosphorus seems more valid, since one would assume that synthesis should be continuing in the organ in spite of the previously collected lipid. On this basis the sphingomyelin synthesis in the material from patients proved to be unremarkable. Specific activity (mμc. of sphingomyelin P^{32}/gm. of tissue) ranged from 0.2 to 2.7 in liver slices of the controls (four hours), with results of 1.3 to 1.5 in the liver of a patient with Niemann-Pick disease. Spleens of controls yielded specific activities of 0.4 to 1.4 and spleen slices from patients with Niemann-Pick disease showed a specific activity of 0.2 to 1.4. The comparative data are given in Table III, for sphingomyelin P^{32}/total lipid P^{32}, showing that it is not possible to demonstrate an increased synthesis of sphingomyelin in this system. Suspensions of washed white blood cells from two other patients with Niemann-Pick disease were also tested, with similar results (Table III).

SUMMARY

A study has been made to investigate the rate at which synthesis of sphingomyelin utilizes tracer inorganic phosphate, with particular attention to the comparison with the whole phospholipid area. *In vivo* rates (twenty-four and forty-eight hours, rats) show sphingomyelin to be somewhat more slowly synthesized, but the ultimate specific activity approaches that of the total phospholipids. With tissue slices, followed for four hours, sphingomyelin production appears to be proceeding at an average of one half to two thirds the rate for the total phospholipids. Liver and spleen slices (and white blood cell suspensions) from patients with Niemann-Pick disease have been tested for *in vitro* lipid synthesis, but the reporting of results is handicapped by the presence of a large pool of previously formed lipid. Specific activity measurements (expressed in terms of unit weight of tissue) do not show evidence for increased synthesis of sphingomyelin in Niemann-Pick disease.

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A Possible Defect in Triglyceride Transport in Idiopathic Hyperlipemia

H. C. MENG, M.D., PH.D.

HYPERLIPEMIA is present in several pathologic and physiologic conditions such as idiopathic hyperlipemia, severe diabetes mellitus, nephrosis, certain liver and pancreatic diseases, von Gierke's disease, starvation, pregnancy, after ingestion of a fatty meal, etc. Remarks will be confined to idiopathic hyperlipemia.

It is well known that the increase in serum lipids in idiopathic hyperlipemia is due predominately to the triglyceride fraction although cholesterol and phospholipids are also elevated.[1–4] We have been interested in studying the metabolic defect which may be responsible for these changes. I should like to comment on the possible sites where the transport of triglycerides could be altered and present some of our preliminary findings with regard to a single specific defect.

POSSIBLE SITES OF ALTERATION

1. Intestinal Absorption. It can be seen in Fig. 1 that the intestinal absorption patterns after ingestion of 80 to 100 μc. of I^{131}-labeled triolein in normal subjects observed in our laboratory were comparable to those reported by Beres et al.[5] and Seller and associates.[6] However, the radioactivity of plasma total lipids measured at the same intervals after ingestion of I^{131}-labeled triolein were higher in idiopathic hyperlipemic patients than those observed in normal subjects (Fig. 2). Unfortunately it is difficult to determine if the elevated radioactivity of plasma lipids in idiopathic hyperlipemic patients is due to an increased rate in intestinal absorption or to a reduced rate of removal from the blood circulation. The disappearance curve following intravenous administration of I^{131}-triolein emulsion should provide information in differentiating these two possibilities.

2. Modification of Absorbed Fat in Intestinal Mucosal Cells. It is known that resynthesis of triglyceride may take place in intestinal mucosal cells.[7,8] Recent evidence has also suggested that some proteins attached to chylomicrons originate in the intestinal mucosa.[9] It is not likely that the ability of intestinal mucosal cells to resynthesize triglyceride is impaired, since our preliminary findings indicated that the appearance of the orally ingested I^{131}-labeled triolein in the plasma is mainly in triglyceride form.[10] However, information is not available with regard to the nature of proteins, cholesterol esters and phospholipids which are linked with chylomicrons in idiopathic hyperlipemia.

3. Modification of Chylomicrons in Intravascular Compartment. Rodbell and associates[9] have shown that the plasma does not contribute to the major portion of the A protein, one of the three proteins associated with chylomicrons. However, further linkage of chylomicrons with other protein(s), may take place in the intravascular compartment. Meng et al.[11] have shown that the mobility of triglyceride emulsion particles measured after incubation with serum or serum globulins was considerably reduced. Histidine, nucleoproteins or protamine exhibit more profound effects

From the Department of Physiology, Vanderbilt University School of Medicine, Nashville, Tennessee.

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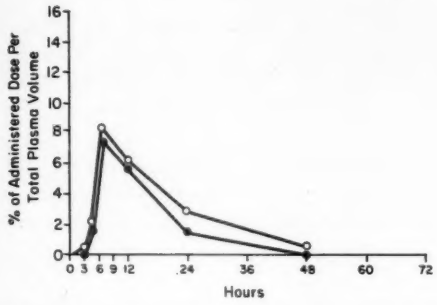

FIG. 1. Fat tolerance curve obtained after oral administration of 100 μc. of I^{131}-labeled triolein in a normal adult male subject showing the radioactivity of plasma total lipids and its rate of disappearance from the blood circulation. ○——○ = whole plasma; ●——● = plasma lipids.

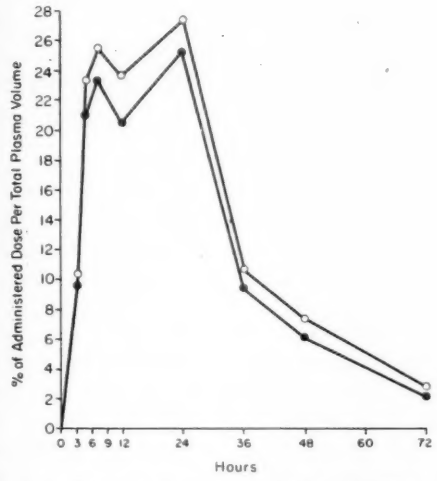

FIG. 2. Fat tolerance curve obtained after oral administration of 50 μc. of I^{131}-labeled triolein in a seven year old girl with idiopathic hyperlipemia showing the elevated radioactivity of plasma total lipids and the reduced rate of its removal from the blood circulation. ○——○ = whole plasma; ●——● = plasma lipids.

on chylomicron mobility. Thus, it is possible that in idiopathic hyperlipemia the nature and composition of these proteins associated with chylomicrons may be altered in the intestinal mucosal cells, in the lacteals or in the blood stream which render the transport of chylomicrons difficult resulting in accumulation in the intravascular compartment. Again, studies of the disappearance of intravenously

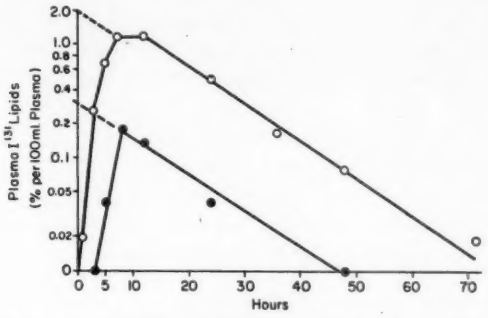

FIG. 3. Showing the disappearance curve of I^{131}-labeled plasma lipids in a normal and an idiopathic hyperlipemic subject. The half-life in the normal subject is seventeen hours and that of the idiopathic hyperlipemic patient is twenty-four hours. ●——● = normal subject (E. S.); ○——○ = idiopathic hyperlipemic subjects (F. M.).

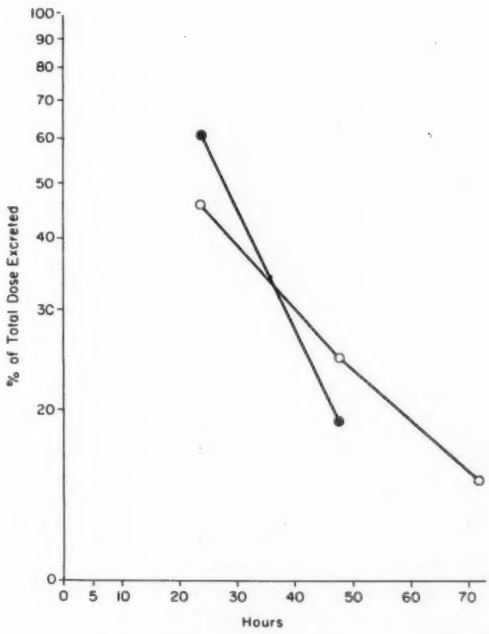

FIG. 4. Showing the delayed urinary excretion of inorganic I^{131} following oral administration of I^{131}-labeled triolein in an idiopathic hyperlipemic subject. ●——● = normal subject (J. B.); ○——○ = idiopathic hyperlipemic subject (B. F.).

administered I^{131}-triolein emulsion might rule out any contribution of the intestinal mucosal cells or passage in the lacteals to the elevation of plasma lipids.

TABLE I
Effect of Heparin on Plasma Lipids

Patient	Time of Blood Withdrawal (Relative to Heparin)	Plasma Lipids			
		Free Fatty Acids (mEq./L.)	Total Fatty Acids (mg. %)	Total Cholesterol (mg. %)	Lipid Phosphorus (mg. %)
B. E.	Before heparin	...	2,030	293	13.5
	30 min. after	...	1,900	286	12.5
F. M.	Before	0.62	2,165	304	18.7
	30 min. after	2.52	2,015	274	15.0
H.	Before	1.04	914	393	11.9
	3 min. after	2.09	918	375	12.5

TABLE II
Effect of Heparin on Plasma Lipoproteins

Patient	Time of Blood Withdrawal (Relative to Heparin)	Plasma Lipoproteins*			
		Prealbumin (%)	Albumin-Alpha (%)	Beta (%)	Total Area
B. E.	Before heparin	0	11.4	88.6	136.5
	30 min. after	0	31.1	68.9	103.0
F. M.	Before	0	5.0	95.0	70.7
	30 min. after	2.9	25.6	71.5	62.8
H.	Before	0	15.6	84.4	51.5
	3 min. after	2.7	33.3	64.0	45.0

* Determined by paper electrophoretic technics.

4. Removal of Chylomicrons from Intravascular Compartment. The accumulation of triglycerides in the intravascular compartment in idiopathic hyperlipemic patients may arise from two causes: (1) decreased rate of removal from the blood stream to other tissues and (2) increased synthesis and accelerated mobilization from tissues to the blood circulation. Results of our studies seem to favor retarded removal.

Thannhauser and Stanley[1] first found that the radioactivity of serum total lipids, measured at intervals after oral ingestion of I^{131}-labeled olive oil, was much higher and the rate of disappearance from the blood stream was considerably slower in idiopathic hyperlipemic patients than in normal persons. Furthermore, the urinary excretion of inorganic I^{131} was lower in idiopathic hyperlipemic patients than in normal subjects. It is readily seen in Figures 3 and 4 that we have confirmed these findings. The uptake of inorganic I^{131} by the thyroid was blocked by the administration of Lugol's solution for two to three days prior to the test in all patients.

The elevation of plasma triglycerides in hyperlipemic patients was apparently not entirely due to the presence of a large triglyceride pool. Crofford and associates[12] demonstrated that the I^{131}-labeled triolein tolerance curve remained high even when the patient was nonhyperlipemic when maintained on a diet furnishing only 1,200 calories per day.

The accumulation of triglycerides in the blood stream is probably not due to the inability of tissue cells to utilize or oxidize the fatty acids. When I^{131}-labeled oleic acid was injected intravenously in the form of an oleic acid-serum albumin complex, the radioactivity disappeared from the blood circulation at the same rapid rate in idiopathic hyperlipemic patients as in normal subjects, with an immediate increase in urinary excretion of inorganic I^{131}.[12]

These results suggest that tissue cells are capable of utilizing the I^{131}-labeled fatty acid when it is made available to them. Studies are in progress to substantiate these observations.

The mechanism of this defect which delays the removal of triglycerides from the blood stream in idiopathic hyperlipemia is not clear. It may be pointed out that the response to intravenous administration of heparin was at least qualitatively normal. It can be seen in Tables I and II that (1) the usual increase in plasma free fatty acids was observed; (2) the expected decrease in plasma total fatty acids occurred in most instances although the changes were not very great; (3) changes in lipoprotein patterns measured by paper electrophoretic technics showed a decrease in total staining area, a decrease in beta-lipoproteins, an increase in albumin-alpha lipoproteins and the appearance of a prealbumin fraction. Other investigators have found similar results in normal and hyperlipemic patients.[13–15] However, it is conceivable that the delayed removal of triglycerides from the intravascular compartment may be due to a lack of "endogenous" lipemia clearing factor or/and increase in clearing factor inhibitor. Hollett and Meng[16] and others[17,18] have found a lipemia-clearing inhibitor in plasma of normal human subjects and animals. Klein and Lever[19] have demonstrated an increase in clearing inhibition by the serum of idiopathic hyperlipemic patients.

6. *Transport of Triglyceride Across the Cell Membrane.* Information is not available concerning the transport of glycerides across the cell membrane in normal or pathologic conditions. It is possible that the uptake of chylomicrons by the cells is impaired in idiopathic hyperlipemic patients. It may also be possible that the synthesis of triglyceride by the tissues is increased or the fat mobilization from the depots is accelerated. Undoubtedly much work remains to be done to unfold the mechanism of elevation of blood triglycerides in idiopathic hyperlipemia.

SUMMARY

The radioactivity of plasma total lipids determined at intervals after oral ingestion of I^{131}-labeled triolein were much higher in idiopathic hyperlipemic patients than in normal individuals. The excretion of inorganic I^{131} in the urine in hyperlipemic patients was slower. The experimental evidence obtained in our laboratory and by other investigators, particularly that of Crofford et al.,[12] suggests that this might be due to a decrease in the removal of triglycerides from the circulation.

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The Serum Lipids and Lipoproteins in Normal and Hyperlipidemic Subjects as Determined by Preparative Ultracentrifugation

Effects of Dietary and Therapeutic Measures. Changes Induced by *In Vitro* Exposure of Serum to Sonic Forces.

ROBERT H. FURMAN, M.D.,* R. PALMER HOWARD, M.D.,† KAPPAGANTULA LAKSHMI, PH.D.‡ AND LEONARD N. NORCIA, PH.D.§

THE 1960 DEUEL CONFERENCE on Lipids has afforded an opportunity for review of some of the disorders of lipid transport and metabolism as well as an opportunity to contribute some previously unreported observations from the authors' recent studies of the ultracentrifugal partition of serum lipids in various "hyperlipemic" and hypercholesterolemic states. Among those studies presented are the relationship between degree of esterification of cholesterol and lipoprotein density, the influence of unsaturated fat feeding and various therapeutic measures on the liproprotein distribution in certain hyperlipidemic disorders and a preliminary evaluation of the effects on lipoproteins of the *in vitro* exposure of serum from normal subjects and patients with various lipid transport abnormalities to sonic forces.

In this paper the term "hyperlipidemia" is employed to designate those disorders in which increased concentrations of cholesterol, phospholipid or "neutral fat" (triglyceride) are noted. The term "hyperlipemia" has become by convention more or less restricted to those conditions in which serum from fasting subjects is found to be lactescent. Hyperlipemia or, more specifically, hyperglyceridemia, may be of the so-called "essential" or idiopathic type or associated with such disorders as diabetes mellitus, hypothyroidism, nephrosis, pancreatitis, acute liver injury, protracted starvation, severe hemorrhage and glycogen storage (von Gierke's) disease. Hypercholesterolemia and hyperphospholipidemia without hyperglyceridemia also may be of the so-called "essential" or idiopathic type, or associated with such disorders as chronic biliary obstruction, hypothyroidism, diabetes mellitus and nephrosis. Marked hyperglyceridemia in the absence of hypercholesterolemia and hyperphospholipidemia is uncommon. On the other hand, marked hypercholesterolemia and hyperphospholipidemia may be noted when serum

From the Cardiovascular Section, Oklahoma Medical Research Institute and the Departments of Medicine and Biochemistry, University of Oklahoma School of Medicine, Oklahoma City, Oklahoma.

* Associate Director of Research, Head of Cardiovascular Section, Oklahoma Medical Research Foundation; † Associate Head of Cardiovascular Section, Oklahoma Medical Research Foundation; ‡Research Assistant, Cardiovascular Section, Oklahoma Medical Research Foundation; present address: Atomic Energy Establishment, Bombay, India; §Biochemist, Cardiovascular Section, Oklahoma Medical Research Foundation; present address: Department of Physiological Chemistry, Temple University School of Medicine, Philadelphia, Pennsylvania.

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TABLE I
Lipoprotein Nomenclature

Authors' Designation	Density Range (g/ml.)	Flotation Rate Designation		Electrophoretic Mobility	Other
		S_f	$-S_{1.21}$		
vld (very low density lipoproteins)	<1.006	20 and above	70 and above	β or "origin" lipid	Includes chylomicrons
ldβ (low density beta lipoproteins)	>1.006 <1.019	12–20	40–70	β	Cohn (method 10) fraction I + III
β lipoproteins	>1.019 <1.063	0–12	25–40	β	Cohn fraction I + III
α lipoproteins	>1.063*	...	0–12	α	Cohn fraction IV + V + VI
Very high density lipoproteins	>1.21	These lipoproteins, sedimentating at 105,400 × g in a solvent density of 1.21 g/ml., contain about 8 per cent of the serum phospholipid and about 2.5 per cent of the serum cholesterol.[10]			

* Lipoproteins undergoing sedimentation in a solvent density of 1.063 g/ml. are considered "α-lipoproteins" for the purposes of this study although, strictly speaking, they are those lipoproteins of density <1.21 >1.063 g/ml.

triglyceride levels are only slightly or moderately elevated.

This relatively simple classification has been amplified by further evaluation of the serum lipids as constituents of lipoproteins wherein the problem is essentially one of classification of the hyperlipoproteinemias.

In the studies to be described a modification[1] of the method of Havel, Eder and Bragdon[2] has been employed for the ultracentrifugal partition of serum lipoproteins. Serum cholesterol has been determined according to the Sperry and Webb modification[3] of the Schoenheimer and Sperry method,[4] lipid phosphorus utilizing wet digestion according to Youngburg and Youngburg[5] and phosphate measurement according to the method of Fiske and Subbarow.[6] The factor of 25 is used to convert lipid P to "phospholipid." Serum fractions obtained after preparative ultracentrifugation were analyzed utilizing semimicro adaptations of the methods just indicated. Serum triglycerides were determined according to the method of Van Handel and Zilversmit[7] and serum "free fatty acids" (FFA) according to the method of Dole.[8] For the sake of brevity and convenience, the five ultracentrifugally defined lipoprotein fractions are referred to as "very low density" (vld), "low density β" (ldβ), "β" lipoproteins, "α" lipoproteins and "very high density" lipoproteins, as indicated in Table I.

SERUM LIPIDS AND LIPOPROTEINS IN "NORMAL" SUBJECTS

The lipid and lipoprotein data from normal men, provided in Table II (and for purposes of comparison in several other tables) represent the results of analyses of twenty-four serum samples from eighteen men varying in age from twelve to fifty-five years, and five serum samples from two men seventy-one and eighty years of age. In none was there evidence of cardiovascular-renal disease, diabetes mellitus or other disorder affecting lipid metabolism and/or transport.

The data for normal women represent analyses of forty-three serum samples from eighteen healthy women ranging in age from fifteen to ninety-six years and divided into pre- and postmenopausal groups. The premenopausal group numbered eleven subjects under forty years of age (mean age twenty-seven years) all with normal ovarian function. The postmenopausal group numbered seven subjects between fifty-one and ninety-six years of age (mean age sixty-nine years).

When lipid data from men are compared

with those obtained from young women, many differences are noted. Men have significantly higher serum triglyceride concentrations and greater mounts of cholesterol and phospholipid in the vld lipoprotein fraction than premenopausal women. There is only very slightly less α lipoprotein lipid (relatively and absolutely) in men than in women, and β and 1dβ lipoprotein lipids show no significant differences. When the 1dβ and vld fractions are combined < 1.019 g/ml. lipoprotein fraction), significantly higher concentrations of these lipids are noted in the men, in agreement with the findings of Havel[2] and Gofman.[9]

When data from younger women are compared with those from older women, significantly greater serum phospholipid and triglyceride concentrations are noted in the postmenopausal subjects, as well as significantly greater amounts of cholesterol in both the vld and the combined 1dβ plus vld lipoprotein fractions. The serum total and free cholesterol concentrations are slightly but not significantly greater in older women. The greater concentrations of triglycerides and lower density lipoproteins in older women, in comparison with premenopausal women, may be related to the increased vulnerability of older women to coronary and cerebral atherosclerosis.

Comparison of the data from men with those from older women reveals significantly higher contents of cholesterol and phospholipid in the vld lipoprotein fractions in men (relatively and absolutely), although the serum triglyceride concentrations in these two groups are almost identical. The serum concentrations of total and free cholesterol and phospholipids are higher in older women as are the amounts of cholesterol and phospholipid in the α, β and 1dβ fractions.

The ratio of cholesterol in α to cholesterol in β lipoprotein (cholesterol α/β ratio) and the ratio of the phospholipid in α and β lipoproteins (phospholipid α/β ratio) were determined. Statistically significant differences were not observed between any two groups but younger men showed lower cholesterol and phospholipid α/β ratios than older men. Younger men also had lower phospholipid α/β ratios than either group of women.

Although only total cholesterol values are listed for the several lipoproteins in Table II, free (unesterified) cholesterol values were determined routinely as well. A progressive increase in the degree of esterification is noted with increasing lipoprotein density and the difference between any two lipoprotein fractions in this respect is statistically significant. (Cohen, Batra and Jones note a greater degree of cholesterol esterification in α and β lipoproteins than in the d < 1.019 g/ml. fraction).[11] Since age and/or sex factors do not appear related to the degree of esterification of cholesterol, the data are presented below for all of the healthy men and women (ages twelve to ninety-six years) in the study:

Per Cent Esterification of Cholesterol, Mean Values ± Standard Error

Whole Serum	Lipoproteins				
	α	β	1dβ	v1d	d < 1.019 g/ml.*
74.5 ± 5.0 (39)	81.3 ± 0.7 (39)	75.6 ± 0.4 (38)	66.1 ± 2.3 (30)	57.8 ± 1.6 (30)	61.3 ± 1.0 (38)

NOTE: Figures in parentheses represent number of subjects.
* vld and 1dβ combined.

HYPERGLYCERIDEMIA ("ESSENTIAL," IDIOPATHIC HYPERLIPEMIA)

The first to recognize this disorder as a disease entity seem to have been Bürger and

TABLE

Normal Subjects: Comparisons Based on

Number of Subjects	Sex and Age (yr.)	Serum Lipids (mg./100 ml.)								
		Cholesterol				C/P (ratio)	G_3 (mg.)	FFA (μEq./L.)	α	
		Total (mg.)	Free (mg.)	Ester (%)	LP × 25 (mg.)				mg.	% total
18	M, 12 to 55	202 ± 8	50 ± 2	75 ± 1	241 ± 7	.84 ± .02§	102 ± 7	622 ± 82	50.0 ± 2.8	(25.5 ± 1.6)
2	M, 71 to 80	181 ± 21	48 ± 4	73 ± 2	233 ± 37	.77 ± .01	73	...	57.0 ± 16.4	(30.3 ± 4.9)
11	F,† 15 to 40	194 ± 13	49 ± 4	75 ± 1	231 ± 11§	.84 ± .02	63 ± 4¶	807 ± 58	53.4 ± 5.4	(27.6 ± 2.2)
7	F, 51 to 96	218 ± 8	58 ± 3	73 ± 1	267 ± 8	.81 ± .01	107 ± 9	838 ± 89	60.8 ± 3.3	(28.0 ± 1.4)
18	M, 12 to 55	202 ± 8	50 ± 2	75 ± 1	241 ± 7	.84 ± .02	102 ± 7¶	622 ± 82	50.0 ± 2.8	(25.5 ± 1.6)
11	F,† 15 to 40	194 ± 13	49 ± 4	75 ± 1	231 ± 11	.84 ± .02	63 ± 4	807 ± 58	53.4 ± 5.4	(27.6 ± 2.2)
18	M, 12 to 55	202 ± 8	50 ± 2‡	75 ± 1‡	241 ± 7§	.84 ± .02	102 ± 7	622 ± 82	50.0 ± 2.8‡	(25.5 ± 1.6)
7	F, 51 to 96	218 ± 8	58 ± 3	73 ± 1	267 ± 8	.81 ± .01	107 ± 9	838 ± 89	60.8 ± 3.3	(28.0 ± 1.4)

TABLE II

Number of Subjects	Sex and Age (yr.)	Lipoprotein Lipid per 100 ml. of Serum (mg. and % of Phospholipid						
		α		β		α/β	pre-β	
		mg.	% total	mg.	% total	Ratio	mg.	% total
18	M, 12 to 55	111.6 ± 4.1	(47.3 ± 1.4)	89.9 ± 4.7	(37.8 ± 1.2)‡	1.29 ± .08	7.9 ± 0.7	(3.4 ± 0.3)
2	M, 71 to 80	119.6 ± 36.1	(51.2 ± 6.9)	79.3 ± 15.9	(34.6 ± 0.9)	1.48 ± .16	5.9 ± 1.8	(2.8 ± 1.3)
11	F,† 15 to 40	116.0 ± 7.2§	(51.2 ± 1.8)	85.8 ± 7.1	(37.8 ± 2.1)	1.45 ± .18	7.8 ± 1.2	(3.7 ± 0.7)
7	F, 51 to 96	139.5 ± 5.4	(52.2 ± 1.5)	96.9 ± 5.3	(36.1 ± 1.1)	1.46 ± .09	11.1 ± 1.1	(4.1 ± 0.4)
18	M, 12 to 55	111.6 ± 4.1	(47.3 ± 1.4)	89.9 ± 4.7	(37.8 ± 1.2)	1.29 ± .08	7.9 ± 0.7	(3.4 ± 0.3)
11	F, 15 to 40	116.0 ± 7.2	(51.2 ± 1.8)	85.8 ± 7.1	(37.8 ± 2.1)	1.45 ± .18	7.8 ± 1.2	(3.7 ± 0.7)
18	M, 12 to 55	111.6 ± 4.1¶	(47.3 ± 1.4)‡	89.9 ± 4.7	(37.8 ± 1.2)	1.29 ± .08	7.9 ±0.7‡	(3.4 ± 0.3)
7	F, 51 to 96	139.5 ± 5.4	(52.2 ± 1.5)	96.9 ± 5.3	(36.1 ± 1.1)	1.46 ± .09	11.1 ± 1.1	(4.1 ± 0.4)

* See test for description of lipoprotein nomenclature.
† Premenopausal women.
‡ $p < 0.05$.
§ $p < 0.02$.
‖ $p < 0.01$.
¶ $p < 0.001$.

Grütz[12] in 1932. The disease is often familial. Lactescence is imparted to the serum by light-scattering lipoprotein particles and hence is a function of both particle size and concentration.

Serum analysis reveals excessive concentrations of triglycerides together with hypercholesterolemia and hyperphospholipidemia, often of marked degree. The term "hyperglyceridemia" is more specific than the popular term "hyperlipemia" but the latter enjoys the prestige of long usage. Alimentary lipemia in healthy subjects is not associated with increase in serum triglyceride levels to the degree commonly seen in this disorder and there is little or no increase in serum cholesterol concentrations.

The serum lipoprotein pattern in hyperglyceridemia is markedly abnormal and varies somewhat according to the method employed for lipoprotein analysis, as is indicated in Table III.

After partial to complete clearing of lactescent serums by centrifugation at 18,000 r.p.m.

II
Age and Sex, Mean Value and Standard Error*

Lipoprotein Lipid per 100 ml. of Serum (mg. and % of serum value)								
Cholesterol								
β		α/β	ldβ		vld		d < 1.019	
mg.	% total	Ratio	mg.	% total	mg.	% total	mg.	% total
120.8 ± 7.4	(59.9 ± 1.9)	.45 ± .05	9.6 ± 0.9	(5.0 ± 0.5)	19.4 ± 1.8	(9.9 ± 0.7)	29.2 ± 2.0	(14.6 ± 1.0)
101.7 ± 17.9	(54.7 ± 2.4)	.55 ± .07	9.5 ± 2.1	(5.3 ± 1.9)	16.5 ± 7.6	(9.7 ± 5.4)	26.1 ± 9.7	(15.0 ± 7.3)
121.9 ± 9.4	(62.9 ± 1.9)	.45 ± .06	9.0 ± 1.1	(4.8 ± 0.6)	9.5 ± 1.4§	(5.2 ± 0.7)	17.7 ± 1.6‖	(9.5 ± 0.9) §
127.9 ± 6.5	(58.6 ± 1.2)	.48 ± .03	15.3 ± 2.8	(6.9 ± 1.0)	13.8 ± 0.7	(6.4 ± 0.4)	29.2 ± 2.9	(13.3 ± 1.0)
120.8 ± 7.4	(59.9 ± 1.9)	.45 ± .05	9.6 ± 0.9	(5.0 ± 0.5)	19.4 ± 1.8¶	(9.9 ± 0.7)¶	29.2 ± 2.0¶	(14.6 ± 1.0)¶
121.9 ± 9.4	(62.9 ± 1.9)	.45 ± .06	9.0 ± 1.1	(4.8 ± 0.6)	9.5 ± 1.4	(5.2 ± 0.7)	17.7 ± 1.6	(9.5 ± 0.9)
120.8 ± 7.4	(59.9 ± 1.9)	.45 ± .05	9.6 ± 0.9	(5.0 ± 0.5)	19.4 ± 1.8‖	(9.9 ± 0.7)¶	29.2 ± 2.0	(14.6 ± 1.0)
127.9 ± 6.5	(58.6 ± 1.2)	.48 ± .03	15.3 ± 2.8	(6.9 ± 1.0)	13.8 ± 0.7	(6.4 ± 0.4)	29.2 ± 2.9	(13.3 ± 1.0)

(*Continued*)

serum value)				Cholesterol/Phospholipid Ratios				
vld		d < 1.019		α	β	ldβ	vld	d < 1.019
mg.	% total	mg.	% total					
28.5 ± 2.5	(12.3 ± 0.9)	35.1 ± 2.3	(14.8 ± 0.8)	0.45 ± .01	1.33 ± .04	1.28 ± .12	0.68 ± .03	0.83 ± .05
23.4 ± 10.3	(11.4 ± 6.5)	29.3 ± 12.1	(14.2 ± 7.8)	0.48 ± .01	1.29 ± .03	1.65 ± .15	0.70 ± .02	0.91 ± .05
16.2 ± 1.6	(7.5 ± 0.8)	24.1 ± 2.6‡	(11.0 ± 1.3)	0.45 ± .02	1.43 ± .03§	1.28 ± .20	0.60 ± .08	0.79 ± .08
20.0 ± 1.0	(7.5 ± 0.3)	31.1 ± 1.9	(11.6 ± 0.6)	0.43 ± .01	1.32 ± .02	1.34 ± .10	0.69 ± .02	0.93 ± .04
28.5 ± 2.5¶	(12.3 ± 0.9)¶	35.1 ± 2.3‖	(14.8 ± 0.8) §	0.45 ± .01	1.33 ± .04§	1.28 ± .12	0.68 ± .03	0.83 ± .05
16.2 ± 1.6	(7.5 ± 0.8)	24.1 ± 2.6	(11.0 ± 1.3)	0.45 ± .02	1.43 ± .03	1.28 ± .20	0.60 ± .08	0.79 ± .08
28.5 ± 2.5‖	(12.3 ± 0.9)¶	35.1 ± 2.3	(14.8 ± 0.8)‖	0.45 ± .01	1.33 ± .04	1.28 ± .12	0.68 ± .03	0.83 ± .05
20.0 ± 1.0	(7.5 ± 0.3)	31.1 ± 1.9	(11.6 ± 0.6)	0.43 ± .01	1.32 ± .02	1.34 ± .10	0.69 ± .02	0.93 ± .04

for one hour, Lever[13] reported a decrease in previously elevated α_2 and β_1 peaks in the electrophoretic pattern and a decrease of 71 per cent in neutral fat, 52 per cent in cholesterol and 37 per cent in phospholipids. Van Eck, Peters and Man[17] found as much as 30 per cent of the serum cholesterol and 50 per cent of the serum neutral fat in the floating creamy layer following serum centrifugation at 18,000 r.p.m. for one to three hours. Thus it is apparent that most of the triglyceride and much of the cholesterol and phospholipid present in excess in this disorder are found with the very low density lipoproteins and chylomicrons.

Serum lipid and liproprotein lipid concentrations in a number of patients with hyperglyceridemia under various conditions of study are listed in Tables IV to VII. Absolute and relative increases in cholesterol and phospholipid of very marked degree are noted in the vld lipoprotein fractions. Analysis of washed chylomicrons separated from the vld fraction of hyperglyceridemic subjects has

TABLE III
Lipoprotein Distribution in "Essential Hyperlipemia" According to Method of Analysis

Method	α Lipoprotein	β Lipoprotein	Other	Laboratory
Free electrophoresis	α_2 elevated	β_1 may be elevated		Lever[13]
Cohn fractionation	α usually normal	Great increase in β (4 ×)		Lever[13]
Paper electrophoresis	Marked depression of α_1 lipoprotein	Markedly elevated	Origin lipid increased	Swahn[14]
	Marked depression of α lipoprotein	Unaltered	Origin lipid increased	Adlersberg[15]
Starch electrophoresis	α_1 absolute and relative decrease α_2 absolute and relative increase	Absolute but not relative increase		Paronetto[16]
Analytical ultracentrifugation in solvent density of 1.063 g/ml.	Not defined by this method	Markedly reduced S_f 0–12 (β) lipoproteins increased S_f 20–100 and marked increase in S_f 100–400 lipoproteins		Gofman[9]

TABLE
Hyperglyceridemia ("essential"

Subjects	Sex and Age (yr.)	Serum Lipids (mg./100 ml.) Cholesterol Total (mg.)	Cholesterol Free (mg.)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Lipoprotein Lipid per 100 ml. of Cholesterol α mg.	α %	β mg.	β %	ldβ mg.	ldβ %	vld mg.	vld %
	M, 12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7
	F, 15 to 40	194 ±13	49 ±4	231 ±11	0.84 ±.02	63 ±4	807 ±58	53.4 ±5.4	27.6 ±2.2	121.9 ±9.4	62.9 ±1.9	9.0 ±1.1	4.8 ±0.6	9.5 ±1.4	5.2 ±0.7
	F, 51 to 96	218 ±8	58 ±3	267 ±8	0.81 ±.01	107 ±9	838 ±89	60.8 ±3.3	28.0 ±1.4	127.9 ±6.5	58.6 ±1.2	15.3 ±2.8	6.9 ±1.0	13.8 ±0.7	6.4 ±0.4
J.L.	M, 47	323	97	343	0.94			26	8	55	17	72	22	179	54
O.W.	M, 47	437	157	449	0.98	968	991	31	7	45	10	43	10	316	73
J.W.	M, 44	342	108	407	0.84	597		27	8	140	40	44	13	133	39
J.S.	F, 53	469	202	690	0.68	2304		20	4	39	8	17	3	417	85
R.W.	M, 21	237	91	367	0.65	738	665	27	11	19	8	29	12	163	69
G.P.	F, 55	334	89	365	0.91	383	417	31	9	159	48	46	14	97	29
L.F.	F, 20	149	52	227	0.66	1208	614	15	10	20	13	5	3	113	74
C.B.J.	M, 2	493	334	1044	0.47	344		4	1	26	5	d < 1.019 g/ml. 471 mg.		94 %	

been carried out by Dr. Anders Gustafson in this laboratory and indicates that almost all of the excess cholesterol and phospholipid present in the vld lipoprotein fraction in this disorder is associated with the chylomicrons. The lipid content of ldβ lipoproteins is normal or moderately increased while that of the α and usually the β lipoproteins is markedly decreased, absolutely and relatively. Similar changes were noted in a patient studied by Havel, Eder and Bragdon.[2] The α/β lipoprotein lipid ratio shows considerable variability because of the wide range of β lipoprotein values which tend to be low when serum triglyceride values are extremely high. The α lipoprotein C/P ratio is often low while that of the vld fraction is at the upper limits of normal or increased. Serum triglyceride levels are, of course, consistently increased while FFA levels are within normal limits, with the exception of subject R.S. (Table VII) whose hyperglyceridemia was associated with "mild diabetes." Normal FFA levels were reported by Dole[18] in three fasting hyperlipemic men.

Of particular interest are the data for subject L.F. (Table IV) whose serum cholesterol and phospholipid levels are in the low or normal range (the abnormal lipoprotein lipid distribution characteristic of idiopathic hyperglyceridemia is readily apparent, however) and C.B.J. (Table IV), a twenty-one month old infant with von Gierke's disease who died two weeks after this sample of blood was obtained.

Examples of hyperglyceridemia secondary

IV
idiopathic hyperlipemia)

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks
Phospholipid												
α		β		ldβ		vld						
mg.	%	mg.	%	mg.	%	mg.	%	α	β	ldβ	vld	
111.6 ±4.1	47.3 ±1.4	89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men
116.0 ±7.2	51.2 ±1.8	85.8 ±7.1	37.8 ±2.1	7.8 ±1.2	3.7 ±0.7	16.2 ±1.6	7.5 ±0.8	0.45 ±.02	1.43 ±.03	1.28 ±.20	0.60 ±.08	Mean value and standard error for eleven premenopausal women
139.5 ±5.4	52.2 ±1.5	96.9 ±5.3	36.1 ±1.1	11.1 ±1.1	4.1 ±0.4	20.0 ±1.0	7.5 ±0.3	0.43 ±.01	1.32 ±.02	1.34 ±.10	0.69 ±.02	Mean value and standard error for seven postmenopausal women
91	29	46	14	40	13	140	44	0.29	1.18	1.80	1.28	Died one month later; coronary thrombosis
115	25	56	12	40	8	254	55	0.29	0.80	1.08	1.24	Mean of two studies; conventional diet
80	21	118	30	31	8	159	41	0.34	1.18	1.42	0.84	Mean of three studies; conventional diet
102	16	46	7	23	4	471	73	0.20	0.85	0.73	0.89	Mean of two studies; conventional diet
119	33	42	12	11	3	184	52	0.23	0.44	2.71	0.88	Hypopituitarism, fibrous dysplasia of bone and hyperglyceridemia
90	26	123	36	36	11	94	27	0.34	1.29	1.28	1.03	Mean of two studies; conventional diet
68	32	30	14	2	1	112	53	0.22	0.66	2.04	1.01	Hyperglyceridemia without hypercholesterolemia
38	4	121	12	d < 1.019 g/ml. 845 mg.		84 %		0.11	0.21	d < 1.019 0.56		von Gierke's disease; died two weeks later

TABLE
Hyperglyceridemia,

Subject and Sex	Age (yr.)	Serum Lipids (mg. per 100 ml.)						Lipoprotein Lipid per 100 ml. of							
		Cholesterol		Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Cholesterol							
		Total (mg.)	Free (mg.)					α		β		1dβ		vld	
								mg.	%	mg.	%	mg.	%	mg.	%
Men	12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7
J.W. (M)	44	342	108	407	0.84	597		27	8	140	40	44	13	133	39
		248	82	338	0.73	502		22	9	90	35	17	7	126	49
		221	70	294	0.75	445	662	20	9	89	39	35	16	82	36
		177	56	249	0.71	342	625	21	12	78	45	18	10	59	33
		201	57	213	0.95	171		35	16	125	58	20	9	34	16

to hypothyroidism and nephrosis are provided in Tables XII and XIII which accompany the discussion of these disorders.

The remarkable serum lipid and lipoprotein pattern of hyperglyceridemia seems characteristic for both primary (i.e., "essential" hyperlipemia) and secondary types.

It is pertinent at this juncture to recall the work of Avigan, Redfield and Steinberg,[19] Shore[20] and Rodbell, Fredrickson and Ono[21] which established that one of the three major proteins present in chylomicrons is a protein characteristic of high density α lipoprotein. Preliminary studies in the authors' laboratory suggest that in serum from normal subjects small amounts of high density lipoproteins are associated with β and 1dβ lipoproteins, and that these lipoproteins may be "freed" or otherwise "dislodged" from β and 1dβ lipoproteins by subjecting the serum to *in vitro* sonic oscillation. A study of the effects of sonic oscillation on serum from patients with various types of hyperlipidemia has been initiated in this laboratory.[22] The changes in lipoprotein cholesterol and phospholipid content in two subjects with hyperlipemia are presented in Table VIII.

The data for subjects R.S. and O.W. (Table VIII) indicate that exposure of serum to sonic oscillation caused appreciable reduction in the cholesterol and phospholipid content of the 1dβ and vld fractions and an equivalent increase in α lipoprotein lipids. It may be that some of the cholesterol in the vld fraction prior to exposure of the serum to sonic oscillation was present as high density lipoprotein in some way attached to, or otherwise associated with, low density lipoproteins and/or chylomicrons. The C/P ratio of α lipoprotein lipid which was low initially increased to normal or slightly greater than normal values following sonic oscillation, suggesting that the high density lipoprotein released from the vld fraction or formed in the serum following sonic oscillation was of the type which presumably is characteristic of the lower density portion of the α lipoprotein continuum. Smaller increases were noted in the cholesterol and phospholipid content of the β lipoprotein fraction, suggesting the possibility that β

v
Effects of Corn Oil Diet

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks		
Phospholipid														
α		β		idβ		vld		α	β	idβ	vld			
mg.	%	mg.	%	mg.	%	mg.	%							
111.6 ±4.1	47.3 ±1.4	89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men		
												Weight (kg.)	Day	
80	21	118	30	31	8	159	41	0.34	1.18	1.42	0.84	84 to 82	1 to 34	2,400 calories, conventional diet; mean of three studies
85	26	65	20	23	7	150	47	0.26	1.38	0.76	0.84	82	44 to 51	2,400 calories, 40 per cent corn oil formula began day 35; mean of three studies
77	26	70	24	29	10	120	40	0.26	1.26	1.21	0.68	82	54 to 76	Same diet; mean of three studies
65	29	62	27	13	6	86	38	0.32	1.26	1.40	0.69	82	100 to 103	Same diet; mean of two studies
72	35	78	37	15	7	43	21	0.49	1.59	1.30	0.78	80	131	Discharged on day 117 on unsaturated fat diet but ate little

lipoproteins were also associated to a lesser degree with the low density lipoproteins. The data do not exclude the possibility that the "redistribution" of lipoprotein lipid caused by sonic oscillation resulted from the formation of lipid micelles which combine with protein to form lipoprotein complexes having flotation and lipid characteristics similar to those of α lipoproteins.

The data from the sonic oscillation experiments are consistent with the evidence from analyses of lipoprotein N- and C-terminal amino acids and protein "fingerprints"[20,21] that α lipoproteins are associated with low density, chylomicron-rich lipoproteins. The cholesterol and phopholipid remaining in the vld fraction following sonic oscillation presumably is that which is an integral part of the low density β lipoproteins and chylomicrons. Oscillation prolonged well beyond two hours does not increase the amount of cholesterol or phospholipid found in the α lipoprotein fraction.

Scanu and Page[60] have presented evidence for the presence of "alpha$_1$ lipoprotein" in purified preparations of chylomicrons. The increase in α lipoprotein reported following heparin-induced clearing by Boyle, Bragdon and Brown[23] and by Brown, Boyle and Anfinsen,[24] or resulting from a diet containing unsaturated fat in diabetic hyperglyceridemic children by Salt and co-workers,[25] may be the result of release of α lipoprotein from chylomicrons as hydrolysis of low density lipoprotein and chylomicron triglyceride occurs and a diminished tendency for α lipoprotein to remain in association with low density lipoproteins or chylomicrons in which the glyceride fatty acids have become more unsaturated as a result of diet.

INFLUENCE OF CORN OIL DIET

The effect on serum lipids and lipoproteins of substituting for the conventional diet a formula composed of skim milk protein (15 per cent of calories) dextrose or Dextri-Maltose (45 per cent of calories) and corn oil (40 per cent of calories) is shown in Table v. Subject J.W. (Table v) was a forty-four year old man with primary hyperglyceridemia, mild angina

TABLE
Hyperglyceridemia, Effects

Subject and Sex	Age (yr.)	Serum Lipids (mg. per 100 ml.)						Lipoprotein Lipid per 100 ml. of							
		Cholesterol		Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Cholesterol							
								α		β		ldβ		vld	
		Total (mg.)	Free (mg.)					mg.	%	mg.	%	mg.	%	mg.	%
Men	12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7
O.W. (M)	47	437	157	449	0.98	968	991	31	7	45	10	43	10	316	73
		386	136	405	0.95	740		31	8	47	12	39	10	266	70
		353	106	393	0.90	834		36	10	57	16	40	11	223	63
		233	77	316	0.74	390		42	18	50	20	39	16	111	46
		214	75	275	0.78	232									
		215	73	295	0.73	223									
		193	57	273	0.71	217	590								
		230	84	307	0.75	363									
		274	87	326	0.84	414		30	11	69	25	40	15	133	49
		371	127	425	0.87	532	600								
		392	135	430	0.91	589	539	39	10	60	15	50	12	251	63
		249	98	336	0.74	424	702	34	14	27	11	44	18	139	57
		275	97	336	0.82	554	466	34	11	40	13	50	17	175	59
		227	91	293	0.77	462	504	28	12	34	14	33	14	139	60
		294	103	361	0.82	942	605	27	10	36	13	38	14	171	63

pectoris and nonspecific electrocardiographic changes. The fasting blood glucose concentrations were normal but intravenous glucose tolerance was slightly impaired.

Corn oil formula feeding (beginning on the thirty-fifth day of the study) which supplied sufficient calories to prevent loss of body weight was associated with reduction in levels of serum cholesterol and phospholipid to normal values. The serum triglycerides fell somewhat but remained well above normal levels. The abnormality in the distribution of lipoprotein remained qualitatively unchanged.

At discharge on the 117 day the patient was placed on an unsaturated fat diet but ate relatively little during the first two weeks following discharge and lost 2 kg. body weight. Serum lipid values at the end of this two week period were almost normal, with further reduction in triglycerides, absolute and relative decrease in the lipid content of the vld fraction and increase in α and β fractions.

Another male patient, O.W., age forty-seven years, with hyperglyceridemia was also studied during feeding with corn oil formula. The study of this patient was complicated because of an upper respiratory infection associated with herpes simplex and fever for twenty-four days. During this febrile period the serum lipids and lipoproteins are of great interest. Data are listed in Table VI. During the first forty-six days of study he received a conventional ward diet in which 40 per cent of the calories were from qualitatively unrestricted fat. Although he gained weight, serum cholesterol and phospholipids fell, vld

VI
of Fever and Corn Oil Diet

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks		
Phospholipid														
α		β		ldβ		vld								
mg.	%	mg.	%	mg.	%	mg.	%	α	β	ldβ	vld			
111.6 ±4.1	47.3 ±1.4	89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men		
												Weight (kg.)	Day	
115	24	56	12	40	9	255	55	0.27	0.80	1.08	1.24	72	4 to 9	2,400 calories, conventional diet; mean of two studies
117	27	55	13	25	6	233	54	0.26	0.85	1.54	1.19	73	48	Same diet
106	28	44	12	50	14	173	46	0.34	1.30	0.81	1.29	75	65	Same diet
133	43	44	14	32	10	103	33	0.32	1.14	1.23	1.07	75	72	2,400 calories, 40 per cent corn oil formula begun day 65
												76	79	2,400 calories, conventional diet resumed day 72; fever and respiratory infection began day 77
												75	85	Low grade fever
												76	89	Low grade fever; herpes labialis
												76	96	Fever subsiding
107	33	57	18	36	11	120	38	0.28	1.22	1.10	1.10	76	101	Afebrile
												76	108	Still receiving 2,400 calorie conventional diet
135	31	57	13	24	6	214	50	0.29	1.05	2.11	1.17	77	112	
104	32	57	17	31	9	139	42	0.33	0.47	1.42	0.99	81	185	2,400 calorie 40 per cent corn oil formula resumed day 153 reduced to 2,000 calories day 166
112	32	45	13	29	8	165	47	0.30	0.89	1.70	1.06	81	192	
102	34	36	12	24	8	137	46	0.27	0.95	1.36	1.01	82	199	
103	29	40	11	35	10	175	50	0.26	0.90	0.85	0.98	84	246	

lipoprotein lipid diminished and there was an increase in α and β lipoprotein lipid. The magnitude of these changes was relatively slight, however, and serum triglyceride levels remained elevated. The corn oil formula was then substituted for the conventional diet and at the end of one week impressive additional improvement was noted in serum lipids and lipoproteins.

Because of the rapidity of improvement in serum lipid and lipoprotein values following use of the corn oil formula, it was decided to resume a conventional diet; but within five days fever and the previously mentioned symptoms developed. Noteworthy is the improvement in serum lipid and lipoprotein patterns during this febrile episode (bringing to mind the experience of Carlson and Olhagen[26] who noted marked decrease in serum glycerides and cholesterol levels during a bout of viral hepatitis in a thirty-eight year old man with hyperlipemia). Defervesence was complete by day 100 and by day 108 the serum lipid and lipoprotein patterns were similar to those characterizing the previous conventional diet period. On resuming the corn oil formula on day 153 the anticipated improvement in serum lipids and lipoproteins occurred. It is noteworthy that the serum triglyceride levels were lower during the bout of fever than when the patient was receiving the corn oil formula although in either instance levels of serum cholesterol and phospholipid attained normal values.

Serum FFA levels remained within normal limits throughout the study other than for

TABLE
Hyperglyceridemia and

Subject and Sex	Age (yr.)	Serum Lipids (mg./100 ml.) Cholesterol Total (mg.)	Cholesterol Free (mg.)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Lipoprotein Lipid per 100 ml. of Cholesterol α mg.	α %	β mg.	β %	ldβ mg.	ldβ %	vld mg.	vld %
Men	12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7
R.S. (M)	57	630	203	686	0.92	2507	1226	12	2	30	5	22	3	564	90
		414	144	447	0.93	950	905	27	7	54	13	32	8	295	72
		401	135	437	0.92	1193	860	23	6	63	16	26	7	277	71
		423	172	504	0.84	1724	952	21	5	27	6	13	3	366	86
		348	136	429	0.81	1427	1075	23	6	24	7	0	0	320	87
		704	243	675	1.05	1906		29	4	57	8	14	2	638	86
		457	155	479	0.95	1201	965	34	7	46	10	33	7	357	76
		286	95	345	0.83	561	791	28	10	77	26	41	14	146	50
		347	119	404	0.87	918	1170	31	8	65	18	36	10	226	64
		385	135	482	0.80	1115		24	6	52	14	20	5	291	75
		415	167	529	0.79	1595	1238	31	7	48	10	8	2	376	81
		392	136	412	0.95	703	747	34	9	84	22	51	13	214	56
		490	184	537	0.91	1809	721	33	7	60	12	34	7	356	74
		550	198	572	0.96	1678		31	5	62	11	35	6	436	78
		680	253	647	1.05	1907	899	30	4	32	5	20	3	595	88
		563	190	605	0.93	1444	1940	29	5	38	7	24	4	470	84

borderline elevation of the initial value.

It is of interest to point out that the C/P ratio of the vld lipoprotein lipids in subject J.W. (Table v) was appreciably and consistently less than that noted in subject O.W. (Table vi) due to the smaller proportion of serum cholesterol found in the vld fraction of the former's serum.

HYPERGLYCERIDEMIA AND "MILD" DIABETES

Hyperglyceridemia, hypercholesterolemia and hyperphospholipidemia with serum lactescence is a well recognized phenomenon in untreated or poorly managed diabetes.[27,28] However, severe hyperlipemia may occur in subjects with "mild" diabetes which does not require insulin and which is associated with normal or only moderately elevated fasting blood glucose levels and little or no glycosuria. Whether such subjects have two disorders, i.e., "essential hyperlipemia *and* mild diabetes,"[29] or hyperlipemia secondary to diabetes, remains to be determined. Of interest in respect to this point is the observation of Williams et al.[30] that cephalin, increased in the plasma of subjects with "essential hyperlipemia," is absent from the plasma of subjects with "diabetic lipemia."

A fifty-seven year old man (R.S.)* (Table VII) with hyperglyceridemia and "mild diabetes," angina pectoris and previous myocardial infarction was studied and the response of the serum lipids and lipoproteins when the

* Three siblings, a daughter and two sons were also studied and none found to have any gross serum lipid abnormality. Two brothers and a nineteen year old son were found to have diminished glucose tolerances. Glucose tolerance was borderline in a twelve year old son and normal in the patient's twenty-seven year old daughter and only living sister.

VII
"Mild Diabetes"

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks		
Phospholipid														
α		β		ldβ		vld								
mg.	%	mg.	%	mg.	%	mg.	%	α	β	ldβ	vld			
111.6 ±4.1	47.3 ±1.4	89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men		
												Weight (kg.)	Day	
99	14	39	6	19	3	530	77	0.12	0.76	1.20	1.06	85	5	1,600 calorie conventional diet began day 1
97	23	40	9	16	4	275	64	0.28	1.34	1.95	1.07	83	28	Calories increased to 1,950 on day 15
87	20	57	13	19	5	265	62	0.26	1.11	1.40	1.04	83	30	1,950 40 per cent corn oil formula began day 31
89	19	26	5	17	4	341	72	0.24	1.04	0.81	1.07	81	60	Corn oil formula; calories increased to 2,150, day 40
83	20	33	8	0	0	293	72	0.28	0.74	...	1.09	81	63	
85	13	32	5	29	4	519	78	0.34	1.78	0.48	1.23	84	150	Uncontrolled diet began day 96
108	23	52	11	15	3	304	63	0.31	0.87	2.14	1.17	83	163	1,980 calories conventional diet began day 150
91	27	61	18	26	8	158	47	0.31	1.27	1.58	0.93	83	192	Same diet, tolbutamide 2 g/d. began day 163
89	23	49	12	20	5	237	60	0.35	1.33	1.83	0.95	84	199	
115	23	46	9	22	5	309	63	0.21	1.13	0.92	0.94	85	206	
99	20	42	8	11	2	359	70	0.31	1.15	0.72	1.05	84	213	
94	23	64	16	30	8	214	53	0.36	1.31	1.67	1.00	85	241	Outpatient; diet uncontrolled since day 192; tolbutamide continued
124	23	42	8	26	5	346	64	0.27	1.42	1.30	1.03	85	262	
116	20	40	7	27	5	398	68	0.27	1.56	1.30	1.10	87	297	
87	14	28	4	23	4	487	78	0.34	1.13	0.89	1.22	86	332	
115	20	36	6	14	3	411	71	0.25	1.06	1.66	1.14	86	386	

corn oil formula was fed and to dietary regulation with and without administration of tolbutamide† is shown in Table VII. The corn oil formula was begun on the thirty-first day and produced relatively little further modification of the serum lipid pattern beyond that already achieved by prior dietary regulation and weight reduction. Limitation of caloric intake together with the administration of tolbutamide seemed to be the most effective means of serum lipid regulation tested during this phase of the study (see day 192, Table VII). A low fat diet was found to be without effect. Intravenous glucose utilization remained decreased throughout the study. Glucosuria was negligible and fasting blood glucose levels were within normal limits during tolbutamide administration, even when many dietary excesses were incurred and serum lipid levels were markedly increased. The effects on serum lipids when corn oil formula feeding was combined with the administration of tolbutamide have not yet been studied in this patient, but 100 mg. of heparin administered subcutaneously twice daily, with or without insulin, failed to control the hyperglyceridemia. Salt and co-workers[25] noted a reduction in β lipoprotein lipid (paper electrophoresis) and in serum cholesterol and phospholipid levels, without change in serum triglyceride concentration, in five diabetic children receiving insulin when placed on a corn oil diet.

The feeding of subjects J.W. and O.W. with the corn oil formula (Tables V and VI) was followed by prompt reduction in serum cholesterol and phospholipid levels to at least close to normal values. In subject R.S.

† Orinase,® The Upjohn Company, Kalamazoo, Michigan.

(Table VII) the response was negligible. Triglyceride values were not appreciably influenced by corn oil. In subject O.W. a diet providing less than 1 per cent of the calories from fat caused a slight increase in serum cholesterol and phospholipid levels above those noted during prolonged feeding with the corn oil formula and no significant change in triglyceride levels. In subject R.S. there was minimal response of the serum lipids to fat restriction of this degree. Fever or weight reduction (i.e., negative caloric balance) lowered serum triglyceride values in subjects J.W. and O.W. In R.S. the lowest triglyceride and cholesterol values occurred while a constant, regular diet and tolbutamide were administered.

Subjects with hypercholesterolemia and slight to moderate hyperglyceridemia (G.P., Table IV; M.S., P.B. and M.T., Table IX) may be classified as "hyperglyceridemics" when there is less than normal cholesterol and phospholipid in absolute as well as relative amounts in the α lipoprotein fraction, and as idiopathic or primary "hypercholesterolemics" when the content of lipids in this fraction is normal or increased in absolute amounts but decreased in relative amounts. The possibility of a "combined lesion," of course, remains a possibility. It is likely that several types of hyperglyceridemia will be noted within the entity "essential hyperlipemia." The presence or absence of abnormal carbohydrate utilization, the serum lipid response to quantitative and/or qualitative alteration of dietary fat and the serum lipid response to the administration of heparin may define several types. The serum lipid response to marked fat restriction in subjects R.S. and O.W. and studies of the incorporation of C^{14} acetate into lipoprotein lipid in the latter subject suggest excessive triglyceride synthesis as a possible mechanism. Injection of heparin provided no evidence of lipoprotein lipase activity in some subjects while others showed "clearing" and marked increase in serum FFA concentrations as evidence of heparin-induced lipoprotein lipase activity. Insufficiency or abnormality of the lipoprotein lipase system therefore may be responsible for hyperglyceridemia in some subjects. The remarkable reduction of excessive levels of 1dβ lipoproteins and chylomicrons during intramuscular androsterone therapy in hyperglyceridemia, recently reported from this laboratory,[31] suggests the possibility of disordered steroid metabolism as yet another etiologic mechanism in this disorder.

The following is a summary of the salient features of the serum lipid and lipoprotein distrubance characterizing hyperglyceridemia or "essential hyperlipemia": (1) There are excessive concentrations of very low density and low density β lipoproteins and chylomicrons causing lactescence. (2) There are excessive amounts of cholesterol and phospholipid, relatively and absolutely, in the density <1.006 g/ml. lipoprotein and chylomicron fraction which may be present, in part at least, as "high density lipoproteins" of a type characterized by an unusually high C/P ratio. Most of these "high density lipoproteins" appear to be associated with chylomicrons. (3) Hyperglyceridemia, hypercholesterolemia and hyperphospholipidemia (with normal or increased C/P ratio) occur as the result of (1) and (2). (4) There is less cholesterol and phospholipid present, absolutely and relatively, in the high density α lipoprotein fraction, due in part at least to (2). (5) There is less cholesterol and phospholipid present, absolutely and relatively, in the β lipoprotein fraction. (6) The C/P ratio of the α and β lipoprotein fractions tends to be low while the vld lipoprotein C/P ratio is normal or increased. (7) Serum FFA concentrations are usually normal.

IDIOPATHIC (PRIMARY OR "ESSENTIAL") HYPERCHOLESTEROLEMIA

In this syndrome, the familial nature of which has long been recognized, the serum characteristically is clear, in spite of high concentrations of cholesterol and phospholipids which usually are less extreme than those noted in idiopathic hyperglyceridemia. Levels of triglyceride are usually normal but may show slight to moderate elevation. Both hyperglyceridemia and hypercholesterolemia of sufficient duration and severity are associated with xanthomas which may differ

TABLE VIII

Milligrams Total (TC) and Free Cholesterol (FC) and Phospholipid (PL) in Lipoprotein Fractions per 100 ml. of Serum and C/P Ratio before and after *In Vitro* Exposure to Sonic Oscillation*

Subject and Disease	Serum				α Lipoprotein				β Lipoprotein				Id β + vld			
	TC	FC	PL	C/P	TC	FC	PL	C/P	TC	FC	PL	C/P	TC	FC	PL	C/P
Ten healthy subjects†																
Mean values																
Presonic	212	53	252	0.84	58	11	123	0.47	126	31	96	1.31	22	9	30	0.73
Postsonic					82	17	145	0.57	102	28	78	1.31	19	8	28	0.68
Mg. gain or loss following sonication					+24	+6	+22		−24	−3	−18		−3	−1	−2	
Hyperglyceridemia																
Subject R.S.																
Presonic	457	145	479	0.95	34	11	108	0.31	46	13	52	0.87	388	127	326	1.19
Postsonic					216	67	268	0.81	53	15	51	1.14	191	66	167	1.14
Mg. gain or loss					+182	+56	+160		+12	+2	−1		−197	−61	−159	
Subject O.W.																
Presonic	351	127	423	0.83	27	4	107	0.25	49	10	72	0.68	278	106	244	1.14
Postsonic					101	28	178	0.57	60	24	82	0.73	183	68	157	1.16
Mg. gain or loss					+74	+24	+71		+11	+14	+10		−95	−38	−87	
Presonic‡	233	77	316	0.73	42		133	0.32	50		44	1.14	154		137	1.12
Postsonic					78		174	0.45	38		23	1.63	125		115	1.09
Mg. gain or loss					+36		+41		−12		−21		−29		−22	
Biliary Cirrhosis																
Subject L.P.																
Presonic	343	169	535	0.64	53	25	166	0.32	268	125	333	0.80	41	13	45	0.91
Postsonic					178	73	293	0.61	126	73	165	0.76	40	15	41	0.96
Mg. gain or loss					+125	+48	+127		−142	−52	−168		−1	+2	−4	
Subject E.S.																
Presonic	891	752	1729	0.52	23		108	0.21	840		1590	0.53	37		40	0.93
Postsonic					481		945	0.51	367		690	0.53	50		46	1.09
Mg. gain or loss					+458		+837		−473		−900		+13		+6	

* Raytheon Model DF 101 10 KC oscillator, two hours at 250 watts.
† The mean of the serum triglyceride content in these healthy subjects was 85 mg. per cent.
‡ This serum from subject O.W. obtained seven days after starting corn oil formula.

TABLE
Primary (Idiopathic)

Subject	Sex and Age (yr.)	Serum Lipids (mg. per 100 ml.) Cholesterol Total (mg.)	Cholesterol Free (mg.)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Lipoprotein Lipid per 100 ml. of Cholesterol α mg.	α %	β mg.	β %	ldβ mg.	ldβ %	vld mg.	vld %
	M, 12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7
	F, 15 to 40	194 ±13	49 ±4	231 ±11	0.84 ±.02	63 ±4	807 ±58	53.4 ±5.4	27.6 ±2.2	121.9 ±9.4	62.9 ±1.9	9.0 ±1.1	4.8 ±0.6	9.5 ±1.4	5.2 ±0.7
	F, 51 to 96	218 ±8	58 ±3	267 ±8	0.81 ±.01	107 ±9	838 ±89	60.8 ±3.3	28.0 ±1.4	127.9 ±6.5	58.6 ±1.2	15.3 ±2.8	6.9 ±1.0	13.8 ±0.7	6.4 ±0.4
M.W.	F, 39	365	94	323	1.13	72	732	64	18	272	75	16	4	11	3
E.M.	F, 46	351	100	365	0.96	147		58	16	181	52	27	8	83	24
M.S.	F, 52	345	91	333	1.04	223	1224	41	11	254	71	14	4	52	14
S.W.	M, 45	315	81	315	1.00	122	671	59	19	197	61	40	12	27	8
D.T.	M, 55	350	91	379	0.92	136		66	18	209	59	34	10	46	13
R.L.	M, 35	405	109	341	1.19	101	615	44	11	329	80	25	6	14	3
		332	82	292	1.14	69	731	52	11	298	86	4	1	5	2
		372	98	324	1.15	81	704	58	15	296	79	11	3	12	3
		376	102	320	1.17	73	623	59	15	298	78	12	3	14	4
		329	77	287	1.15	82	757	48	15	267	81	7	2	6	2
												d < 1.019 g/ml.			
												mg.		%	
P.B.	F, 39	334	93	326	1.02	298		47	14	244	73	44		13	
		281	78	280	1.00	129		40	14	200	70	45		16	
		427	124	380	1.12	115		51	12	305	75	55		13	
M.T.	F, 43	468	132	417	1.12	200		33	7	309	64	139		29	
		431	118	359	1.20	118		39	9	317	74	72		17	
		525	139	431	1.21	206		40	7	350	64	156		29	

somewhat in respect to type and distribution in the two disorders (e.g., xanthelasmas and tendon xanthomas are much more common in hypercholesterolemia, while tuberous xanthomas and "eruptive xanthomas" are more common in hyperlipemia). Gofman[9] and Boyle[32] suggested that the nature and distribution of these xanthomas might reflect patterns of distribution of β and lower density lipoproteins which are characteristic of the disorders. Arcus senilis is often seen in idiopathic hypercholesterolemia, rarely if ever in hyperglyceridemia. "Retinal lipemia" is not uncommon in hyperglyceridemia, especially hyperglyceridemia accompanying diabetes and nephrosis.

The absence of serum lactescence in uncomplicated primary hypercholesterolemia de-

IX
Hypercholesterolemia

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks
Phospholipid												
α		β		ldβ		vld						
mg.	%	mg.	%	mg.	%	mg.	%	α	β	ldβ	vld	
111.6 ±4.1	47.3 ±1.4	89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men
116.0 ±7.2	51.2 ±1.8	85.8 ±7.1	37.8 ±2.1	7.8 ±1.2	3.7 ±0.7	16.2 ±1.6	7.5 ±0.8	0.45 ±.02	1.43 ±.03	1.28 ±.20	0.60 ±0.8	Mean value and standard error for eleven premenopausal women
139.5 ±5.4	52.2 ±1.5	96.9 ±5.3	36.1 ±1.1	11.1 ±1.1	4.1 ±0.4	20.0 ±1.0	7.5 ±0.3	0.43 ±.01	1.32 ±.02	1.34 ±.10	0.69 ±.02	Mean value and standard error for seven postmenopausal women
121	38	176	54	12	3	15	5	0.53	1.55	1.33	0.73	Mean of two studies; conventional diet
134	36	140	38	21	6	75	20	0.43	1.29	1.27	1.11	Conventional diet
105	32	152	46	17	5	57	17	0.39	1.68	0.85	0.91	Mean of two studies; conventional diet
118	39	133	43	27	9	28	9	0.50	1.48	1.46	0.95	Conventional diet
153	41	160	42	19	5	45	12	0.43	1.31	1.76	1.03	Mean of three studies
99	30	198	61	14	4	15	5	0.44	1.67	1.80	0.92	Mean of three studies; slight fat restriction
89	32	166	60	11	4	11	4	0.58	1.80	0.32	0.46	After three months less than 10 per cent fat calories
116	37	175	55	11	4	13	4	0.50	1.68	0.93	0.96	After four and one-half months less than 10 per cent fat calories
103	33	193	61	7	2	14	4	0.57	1.54	1.73	1.00	Conventional diet, 40 per cent of calories from fat, 2,200 calories; mean of two studies
94	34	172	62	3	1	8	3	0.51	1.55	1.91	0.75	2,200 calories, 40 per cent corn oil formula for two weeks; mean of two studies
				d < 1.019 g/ml.								
				mg.		%				d < 1.019 g/ml.	Weight (kg.)	Day	
101	33	168	54	41		13		0.47	1.44	1.06	52	1 to 15	Conventional diet, mean of four studies
95	35	134	50	39		15		0.42	1.50	1.15	52 to 53	35 to 45	Corn oil formula since day 17; mean of three studies
103	28	218	59	50		13		0.50	1.40	1.09	54	120	Conventional diet since day 57
83	21	213	53	102		26		0.40	1.45	1.36	62	1 to 8	Conventional diet, mean of three studies
93	26	210	59	54		15		0.42	1.51	1.33	62	33 to 38	Corn oil formula since day 17, mean of two studies
88	21	232	54	109		25		0.45	1.51	1.43	62		Conventional diet for eleven days

notes a paucity of low density, light-scattering lipoproteins and chylomicrons. Ultracentrifugal studies by Gofman[9] in subjects with "xanthoma tendinosum" indicated that the increase in serum lipoproteins was limited to the S_f 0–12 or β lipoprotein fraction. Cohn fractionation studies by Lever[13] revealed greatly increased amounts of cholesterol and phospholipid in the β fraction and normal or less than normal amounts in the α fraction. Hood and Angervall[33] also reported increased cholesterol in the β lipoprotein fraction and less than normal amounts in the α lipoprotein fraction in women over fifty years of age, and in men, following Cohn fractionation studies. In younger women with this disorder they found no absolute reduction in α lipoprotein cholesterol. Paper electrophoresis[15] and zone

TABLE
Hypercholesterolemia Due

Subject	Sex and Age (yr.)	Serum Lipids (mg. per 100 ml.)							Lipoprotein Lipid per 100 ml. of							
		Cholesterol		Ester (%)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Cholesterol							
		Total (mg.)	Free (mg.)						α		β		ldβ		vld	
									mg.	%	mg.	%	mg.	%	mg.	%
	F, 15 to 40	194 ±13	49 ±4	75 ±1	231 ±11	0.84 ±.02	63 ±4	807 ±58	53.4 ±5.4	27.6 ±2.2	121.9 ±9.4	62.9 ±1.9	9.0 ±1.1	4.8 ±0.6	9.5 ±1.4	5.2 ±0.7
	F, 51 to 96	218 ±8	58 ±3	73 ±1	267 ±8	0.81 ±.01	107 ±9	838 ±89	60.8 ±3.3	28.0 ±1.4	127.9 ±6.5	58.6 ±1.2	15.3 ±2.8	6.9 ±1.0	13.8 ±0.7	6.4 ±0.4
L.P.	F, 64	776	648	17	1509	0.51	188	890	15	2	616	77	108	13	61	8
		700	586	16	1344	0.52	168	967	16	2	643	94	14	2	12	2
		408	172	58	558	0.73	91	671	62	15	321	76	17	4	22	5
		357	186	48	549	0.65	108	949	52	14	284	78	17	5	12	3
E.S.	F, 40	1466	1330	9	3069	0.48	302		37	2	1411	95	14	1	25	2
		1233	1041	16	2492	0.50	337		23	2	1143	93	24	2	41	3
		1017	773	24	1872	0.54	349	694	20	2	969	90	39	4	43	4
		745	593	20	1477	0.50	157	829	40	5	666	88	27	4	25	3
C.D	F, 60	393	301	23	732	0.53			38	9	365	88	(d < 1.019; 13 mg. 3%)			
		325	286	12	593	0.55			25	7	303	88	12	3	6	2
		153	120	21	329	0.46			16	10	139	87	3	2	2	1
		124	96	22	270	0.46			25	19	96	73	6	4	5	4
		162	131	15	327	0.49			19	11	138	82	7	4	4	3
		90	82	9	233	0.39	66									
		110	92	18	237	0.46										

or starch electrophoresis[16, 34] have revealed normal or decreased α lipoproteins and increased β lipoproteins.

Serum lipid and lipoprotein data from seven patients with primary "essential" hypercholesterolemia studied in this laboratory are presented in Table IX. The data are similar to those reported from a case of "xanthoma tendinosum" studied by Havel, Eder and Bragdon.[2]

The data in Table IX indicate that the excess serum cholesterol and phospholipid in this disorder are in the β (density $> 1.019 < 1.063$ g/ml.) fraction. While the β lipoprotein lipids are increased, α lipoprotein lipids are usually normal or slightly increased in amount in terms of actual content of cholesterol and phospholipid, but are relatively reduced because of the high total serum concentration of these lipids. Hence the α/β lipoprotein lipid ratio is low. The C/P ratios of serum and of the several lipoprotein fractions may be normal but are often increased. The serum triglyceride concentration is usually normal but may be slightly elevated; FFA concentrations are within normal limits. Thus this disorder might be designated "hyperbetalipoproteinemia."

The close relationship between carbohydrate and lipid metabolism in relation to primary hypercholesterolemia and hyperglyceridemia has been emphasized again recently by Wad-

X
to Biliary Cirrhosis

Serum (mg. and % of serum value)								Cholesterol/Phospholipid Ratio				Remarks			
Phospholipid															
α		β		ldβ		vld									
mg.	%	mg.	%	mg.	%	mg.	%	α	β	ldβ	vld				
116.0 ±7.2	51.2 ±1.8	85.8 ±7.1	37.8 ±2.1	7.8 ±1.2	3.7 ±0.7	16.2 ±1.6	7.5 ±0.8	0.45 ±.02	1.43 ±.03	1.28 ±.20	0.60 ±.08	Mean value and standard error for eleven premenopausal women			
139.5 ±5.4	52.2 ±1.5	96.9 ±5.3	36.1 ±1.1	11.1 ±1.1	4.1 ±0.4	20.0 ±1.0	7.5 ±0.3	0.43 ±.01	1.32 ±.02	1.34 ±.10	0.69 ±.02	Mean value and standard error for seven postmenopausal women			
												Weight (kg.)	Direct Bilirubin (mg.)	Day	
88	6	1166	77	139	9	113	8	0.17	0.53	0.78	0.54	35	9.0	6	Conventional diet
69	5	1228	92	9	1	21	2	0.23	0.52	1.60	0.56	35		8 to 17	Mean of three studies
161	29	348	62	9	2	39	7	0.39	0.92	2.04	0.57	35	6.0	62	40 per cent corn oil formula began on day 17
169	31	344	63	17	3	19	3	0.31	0.83	0.98	0.62	35	7.8–5.3	86 to 171	Still on corn oil formula; mean of four studies
93	3	2850	95	20	1	34	1	0.40	0.50	0.69	0.74	64	4.0	1	Conventional diet, phenylbutazone for "arthritis"
97	4	2326	93	34	1	57	2	0.24	0.49	0.71	0.71	62	5.0	20	Same diet, no phenylbutazone for one week
96	5	1740	91	42	2	41	2	0.21	0.56	0.93	1.04	63	3.9–2.6	33 to 40	Conventional diet 40 per cent fat since day 20, mean of three studies
149	10	1226	86	27	2	26	2	0.27	0.54	1.00	0.96	62	2.1–1.5	85 to 161	Corn oil formula since day 54; mean of four studies
128	17	580	79	(d < 1.019; 26 mg. 4%)				0.30	0.63	0.52		60	14.8	2/24/58	Outpatient status; low fat intake
82	12	550	82	22	3	20	3	0.30	0.55	0.54	0.31	55	18.6	7/22/58	
70	22	225	71	9	3	11	4	0.23	0.62	0.34	0.19	53		1/19/59	
57	22	188	71	11	4	8	3	0.44	0.51	0.52	0.68	54	21.4	6/29/59	
63	20	227	74	12	4	6	2	0.30	0.61	0.60	0.73	54	28.6	10/5/59	
												50	13.5	4/25/60	
												51		4/28/60	

dell, Geyer, Hurley and Stare[35] who reported that eighteen of twenty subjects with idiopathic hypercholesterolemia and each of five subjects with essential hyperlipemia had impaired glucose tolerance. The majority of patients also exhibited increased insulin sensitivity. These workers suggested that "relative pancreatic insufficiency" resulted in a "diabetic-like" state which led to elevated serum lipid levels in these patients. We have evaluated glucose tolerance and found it impaired in four of eight subjects with hyperlipemia, borderline in the other four. Of six subjects with hypercholesterolemia, three were normal, two were borderline and one revealed impaired tolerance.

EFFECTS OF CORN OIL FORMULA FEEDING

Reduction in serum cholesterol levels has been reported[36] following the substitution of corn oil for all dietary fat in patients with primary hypercholesterolemia. Table IX indicates the nature and degree of the serum lipid and lipoprotein abnormalities in two sisters (P.B., thirty-nine years of age and M.T., forty-three years of age) with primary familial hypercholesterolemia and the response of the serum lipids to the substitution of the corn oil formula for the regular diet. Our efforts to reduce serum cholesterol levels in these subjects by dietary means have been only moderately successful and our experience in this respect is not unique.[37] However, serum cholesterol and

TABLE XI
Total Cholesterol and "Lipid Phosphorus" Content of Whole Serum and d > 1.21 g/ml. Fraction

Subjects Mean Values	Serum Values (mg. %)		Cholesterol (mg.) in d > 1.21	Per Cent Serum Cholesterol in d > 1.21	Lipid P (mg.) in d > 1.21 g/ml.	Per Cent of Serum Lipid P in d > 1.21
	Cholesterol	Lipid P				
16 healthy women*	205.7 ± 37.9	9.81 ± 1.45	4.19 ± 1.77	2.0 ± 0.7	1.13 ± 0.14	11.7 ± 2.1
Biliary cirrhosis						
C.D. 7/58	341	26.63	3.6	1.1	0.88	3.3
1/59	154	13.16	2.1	1.3	0.89	6.8
6/29	132	10.79	3.6	2.8	0.92	8.5
L.P. (mean of 4)	727	54.90	5.4	0.7	1.10	2.0
E.S. (mean of 5)	1180	91.69	6.3	0.5	1.25	1.4

* Standard deviation of the mean is shown for data on healthy women.

phospholipid levels fell slightly during the period of corn oil formula feeding and rose when the regular diet was restored. There was no qualitative change in the abnormality of lipoprotein distribution.

The effect of sonic oscillation on serum from subjects with idiopathic hypercholesterolemia is under study. Preliminary results suggest that the lipid content of β hyperlipoproteins in essential or idiopathic hypercholesterolemia is not enhanced by the presence of higher or lower density lipoproteins.

The following is a summary of the salient features of the serum lipid and lipoprotein disturbance characterizing uncomplicated idiopathic, primary or "essential" hypercholesterolemia: (1) Excessive concentrations of β lipoproteins without serum lactescence; (2) moderate to marked hypercholesterolemia and hyperphospholipidemia as the result of (1); (3) normal degree of cholesterol esterification; (4) relative decrease in high density α lipoprotein lipid; actual content of cholesterol and phospholipid in this lipoprotein fraction usually normal or increased; (5) normal or increased lipid content of 1dβ and vld lipoproteins; relative to serum values, the content of these fractions shows no constant pattern; (6) increased serum C/P ratio; this is generally true also of the individual lipoprotein fractions; (7) serum triglyceride concentration usually normal but may be elevated; (8) normal FFA concentrations.

HYPERCHOLESTEROLEMIA SECONDARY TO BILIARY OBSTRUCTION

The serum lipid and lipoprotein pattern in biliary obstruction of either intraphepatic or extraphepatic type is unique. Prior to the onset of hepatic failure hypercholesterolemia and hyperphospholipidemia develop which may be extreme, i.e., in excess of 2,000 mg. per 100 ml. The increment in serum cholesterol is mainly confined to unesterified (free) cholesterol, while esterified cholesterol values usually are normal or low. The per cent esterification of serum cholesterol therefore is often extremely low. The increment in phospholipid is usually relatively greater than that of the cholesterol, hence a low C/P ratio is characteristic. High density lipoproteins are reduced in amount or totally absent in this disorder, when analyzed by zone electrophoresis,[34] analytic ultracentrifugation[39] or differential preparative ultracentrifugation.[2,38,40] Following Cohn fractionation, however, large amounts of lipid are found in fraction IV + V + VI,[40] the fraction containing α lipoproteins of serum from healthy subjects.

In Table x are presented serum lipid and lipoprotein data from three women with chronic primary biliary obstruction, two of whom (C.D. and E.S.) had pericholangiolytic

biliary cirrhosis of the type described by MacMahon,[41,42] the other (L.P.) had biliary cirrhosis most closely resembling a post-necrotic type.

It is evident from inspection of the data in Table x that biliary obstruction is associated with great increase, relative as well as absolute, of cholesterol and phospholipid in the β lipoprotein fraction and decrease in the α fraction. Furthermore the C/P ratio of the β lipoprotein lipids is extremely low. The degree of esterification of cholesterol is very low in both α and β lipoprotein fractions as well as whole serum but tends to be higher in fractions of density <1.019 g/ml. (not shown in table).

The decrease in serum lipid values in subject C.D. first noted on January 19, 1959 (Table x) was spontaneous and taken as evidence of beginning failure in this parameter of liver function. Death occurred twenty-one months later. It is noteworthy that the serum lipoprotein "profile" remained essentially unaltered although serum cholesterol and phospholipid levels were considerably reduced.

In 1955 Havel, Eder and Bragdon[2] directed attention to the "lipid phosphorus" which could be recovered from the very high density (>1.21 g/ml.) fraction of human serum. In a subsequent report from the authors' laboratory[10] it was noted that approximately 8 per cent of the lipid phosphorus and 2.5 per cent of the cholesterol of whole serum could be accounted for in this very high density fraction in twenty-six serum samples obtained from sixteen healthy subjects. Analyses of sixty-three serum samples obtained from a young woman with pericholangiolytic biliary cirrhosis indicated that only about 0.27 mg. or 0.5 per cent of the 54.5 mg. of lipid phosphorus in the serum was present in this fraction. Phillips[43] confirmed the figure of 8 per cent for normal subjects and reported that about half of this phospholipid existed as lysolecithin which in turn comprised about half of the total lysolecithin of serum. It is of interest that the phospholipid content of the very high density fraction of sera from the three women with biliary cirrhosis is only relatively reduced (Table xi) and may be normal in absolute amount. Serum from these three patients contained some α lipoproteins while in the serum from the patient with biliary cirrhosis described previously[10] no α lipoproteins were discernible by analytic (refractometric) ultracentrifugation.

The serum lipoproteins in biliary cirrhosis have been characterized further by Russ, Raymunt and Barr[40] who noted three atypical lipoproteins each with lower than normal peptide:lipid and cholesterol:phospholipid ratios. It is evident that the serum lipoproteins in this disorder are unique in respect to their distribution and composition.

We have subjected hypercholesterolemic serum from patients with biliary obstruction to *in vitro* sonic oscillation (Table viii). A considerable amount of cholesterol and phospholipid was "released" from the $d > 0.019 < 1.063$ g/ml. (β) fraction by this procedure and comparable gain in the lipid content of α lipoprotein was noted. This suggests an abnormal association of high density lipoproteins with "β lipoproteins" in this disorder. The C/P ratio of these high density lipoproteins is greater than that characteristic of "normal" α lipoproteins.

Walker, Flynn and Kinsell[44] lowered the serum cholesterol concentration in a patient with biliary cirrhosis from levels of 1,400 mg. to approximately 400 mg. per 100 ml., utilizing a diet containing ethyl linoleate. The response of the serum lipids and lipoproteins in two of the women with biliary cirrhosis (L.P. and E.S.) to substitution of the corn oil formula for the regular diet is indicated in Table x. Noteworthy is the fall in serum unesterified cholesterol and phospholipid values and the increase in esterified cholesterol and hence in the degree of esterification in both patients. The α lipoprotein increased appreciably in patient L.P. In patient E.S. there was impressive reduction in the degree of icterus.

The following is a summary of the salient features of the serum lipid and lipoprotein disturbance characterizing hypercholesterolemia secondary to biliary cirrhosis: (1) Moderate to marked increase in cholesterol and phospholipid content of "β" lipoproteins (density $>$ 1.019 $<$ 1.063 g/ml.); in this

TABLE
Hypothy-

Subject	Sex and Age (yr.)	Serum Lipids (mg. per 100 ml.) Cholesterol Total (mg.)	Cholesterol Free (mg.)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Lipoprotein Lipid per 100 ml. of Serum (mg. and Cholesterol α mg.	α %	β mg.	β %	1dβ mg.	1dβ %	vld mg.	vld %	α mg.	α %
	M, 12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7	111.6 ±4.1	47.3 ±1.4
	F, 51 to 96	218 ±8	58 ±3	267 ±8	0.81 ±.01	107 ±9	838 ±89	60.8 ±3.3	28.0 ±1.4	127.9 ±6.5	58.6 ±1.2	15.3 ±2.8	6.9 ±1.0	13.8 ±0.7	6.4 ±0.4	139.5 ±5.4	52.2 ±1.5
G.C.	F, 50	544	146	426	1.28	114	865	66	12	446	79	31	6	18	3	124	28
O.R.	M, 40	421	113	367	1.13	283	596	30	7	215	52	49	12	123	30	85	23
S.B.	M, 54	522	142	451	1.16	247		50	10	314	61	56	11	94	18	106	24
		176	41	199	0.88	68		40	23	121	68	5	3	10	6	87	44
P.Y.	F, 59	633	158	486	1.30			26	4	368	59	130	21	98	16	68	15
H.S.	M, 58	397	118	368	1.08			17	6	157	42	78	20	120	32	65	10
		200	60	206	0.97			15	7	129	61	20	10	47	22	60	29
		132	34	164	0.80			31	22	72	53	11	8	23	17	67	43
C.J.	F, 64	751	315	747	1.01												
	67	319	85	370	0.86	408		42	13	100	31	53	16	127	40	117	26
	68	249	68	287	0.87	223	972	64	26	106	43	26	11	50	20	125	43
M.C.	F, 57	523	170	568	0.93	956	640	34	7	112	21	73	14	308	58	111	20
		243	78	306	0.80	477	1046	25	10	84	35	20	8	114	47	75	25
O.W.*	M, 47	437	157	449	0.98	968	991	31	7	45	10	43	10	316	73	115	25

* "Essential hyperlipemia" (see Table IV).

fraction the C/P ratio is low and the degree of esterification of cholesterol is low; (2) moderate to extreme hypercholesterolemia and relatively greater hyperphospholipidemia; serum C/P ratio and degree of esterification of cholesterol less than normal, due to (1); (3) significant amounts of cholesterol and phospholipid in the density > 1.019 < 1.063 g/ml. fraction may be in the form of high density lipoproteins, associated with or bound in some way to "β" lipoproteins and accounting, at least in part, for (1); (4) moderate to extreme reduction, relative and absolute, of α lipoprotein lipid (except when assayed by Cohn method X fractionation technic); (5) normal or increased content of lipid in the 1dβ and vld fractions, although content relatively less because of the high serum cholesterol and phospholipid values; (6) normal or moderate increase in serum triglyceride concentrations; (7) normal FFA concentrations; (8) serum icteric and usually clear.

HYPERLIPIDEMIA SECONDARY TO HYPOTHYROIDISM

The hypercholesterolemia, hyperphospholipidemia and increased C/P ratio accompanying hypothyroid states are well known. Gofman[9] reported marked increase in S_f 0–12 (β) and minimal increase in S_f 12–20 (1dβ) lipoproteins in two myxedematous sub-

XII
roidism

% of serum value)											
Phospholipid						Cholesterol/Phospholipid Ratio				Remarks	
β		ldβ		vld							
mg.	%	mg.	%	mg.	%	α	β	ldβ	vld		
89.9 ±4.7	37.8 ±1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men	
96.9 ±5.3	36.1 ±1.1	11.1 ±1.1	4.1 ±0.4	20.0 ±1.0	7.5 ±0.3	0.43 ±.01	1.32 ±.02	1.34 ±.10	0.69 ±.02	Mean value and standard error for seven postmenopausal women	
										Weight (kg.)	
273	63	19	4	21	5	0.53	1.63	1.63	0.86	105	Postoperative hypothyroidism; no thyroid treatment; mean of three studies
153	42	35	10	89	25	0.35	1.41	1.40	1.38	77	Hypothyroidism since I^{131} treatment nine months previously; no thyroid treatment; mean of four studies
221	51	33	8	74	17	0.47	1.41	1.72	1.12	80	Spontaneous hypothyroidism; no thyroid treatment; mean of three studies
88	45	4	2	17	9	0.46	1.37	1.35	0.57	75	Tri-iodothyronine 25 to 100 μg./d. for forty days; mean of four studies
234	50	96	21	65	14	0.38	1.57	1.35	1.52	77	Spontaneous hypothyroidism; no thyroid treatment; mean of two studies
136	43	53	17	95	30	0.26	1.15	1.47	1.26	112	Spontaneous hypothyroidism; no thyroid treatment; mean of two studies
84	40	18	9	47	22	0.25	1.53	1.11	0.98	107	Tri-iodothyroacetic acid for sixteen days, 2 to 4 mg./d.
49	32	7	5	31	20	0.46	1.47	1.57	0.74	97	Mean of three sera obtained between day 44 and 78 of treatment at 4 mg./d.
										87	Spontaneous hypothyroidism; no thyroid treatment; mean of four studies
82	25	35	11	125	38	0.36	1.22	1.51	1.02	80	Thyroid, U.S.P. 300 to 180 mg./d. for thirty-six months; mean of three studies
89	31	19	7	55	19	0.51	1.19	1.37	0.91	82	Thyroid, U.S.P. 180 mg./d. continued for an additional four months; mean of two studies
110	19	65	12	274	49	0.31	1.02	1.09	1.12	70	Spontaneous hypothyroidism; no thyroid treatment; mean of three studies
66	22	19	6	144	47	0.34	1.26	1.20	0.79	66	Tri-iodothyronine 50 to 100 μg./d. for thirty-two days; mean of three studies
56	12	40	8	254	55	0.29	0.80	1.08	1.24	72	Hyperglyceridemia; mean of two studies

jects. Jones, Cohen and Corbus[45] found similar increases in β and ldβ lipoproteins in the serum of seventeen patients with hypothyroidism and little or no reduction in α lipoprotein concentrations in comparison with hyperthyroid subjects. Kunkel and Slater,[34] employing starch or zone electrophoresis, reported a low α lipoprotein peak in one patient with hypothyroidism. Jones[45] noted a higher β lipoprotein concentration in hypothyroid subjects than in subjects with primary hypercholesterolemia. Malmros and Swahn[46] also noted increased β lipoprotein concentrations in hypothyroidism. Thyroid administration caused diminution in β lipoprotein cholesterol and increase in α lipoprotein cholesterol.[46,47]

Data representative of the serum lipid and lipoprotein patterns in serum hypothyroid subjects are presented in Table XII. It is evident that hypothyroidism is associated with a "hyperbetalipoproteinemia" similar to that seen in primary hypercholesterolemia (Table IX). Of great interest is the finding that an impressive number of hypothyroid subjects are hyperglyceridemic as well as hypercholesterolemic (e.g., subjects C.J. and M.C.) and one is tempted to postulate two types of hyperlipidemia secondary to hypothyroidism, i.e., a hypercholesterolemic-hyperglyceridemic type (subject M.C.) and a

TABLE
Neph-

Subject	Sex and Age (yr.)	Serum Lipids (mg. per 100 ml.) Cholesterol Total (mg.)	Cholesterol Free (mg.)	Phospholipid (mg.)	C/P Ratio	Triglyceride (mg.)	FFA (μEq./L.)	Lipoprotein Lipid per 100 ml. of Serum (mg. and Cholesterol α mg.	α %	β mg.	β %	ldβ mg.	ldβ %	vld mg.	vld %	α mg.	α %
	M, 12 to 55	202 ±8	50 ±2	241 ±7	0.84 ±.02	102 ±7	622 ±82	50.0 ±2.8	25.5 ±1.6	120.8 ±7.4	59.9 ±1.9	9.6 ±0.9	5.0 ±0.5	19.4 ±1.8	9.9 ±0.7	111.6 ±4.1	47.3 ±1.4
	F, 15 to 40	194 ±13	49 ±4	231 ±11	0.84 ±.02	63 ±4	807 ±58	53.4 ±5.4	27.6 ±2.2	121.9 ±9.4	62.9 ±1.9	9.0 ±1.1	4.8 ±0.6	9.5 ±1.4	5.2 ±0.7	116.0 ±7.2	51.2 ±1.8
E.R.	M, 12	867	203	709	1.22	274		89	11	601	72	46	5	100	12	161	23
		508	124	480	1.05	271		58	13	304	66	31	7	66	14	135	29
J.F.	F, 18	361	97	337	1.07	178		49	13	232	63	22	6	65	18	112	34
		209	49	231	0.91	97		47	22	130	60	10	5	27	13	109	47
S.S.	M, 40	683	194	577	1.19	280		50	7	528	79	39	6	53	8	121	21
		481	160	481	1.00	223		67	13	355	71	5	1	77	15	159	33
P.W.	M, 44	254	66	276	0.91	200		41	15	129	49	31	12	63	24	92	35
S.M.	F, 13	361	119	384	0.95	651	429	28	8	72	20	39	11	224	61	83	23
		769	237	557	1.40	1101	210	12	2	147	20	75	10	510	68	46	8
O.W.*	M, 47	437	157	449	0.98	968	991	31	7	45	10	43	10	316	73	115	25

* "Essential hyperlipemia" (see Table IV).

hypercholesterolemic-normoglyceridemic type (subject G.C.). While the severity of the hypothyroidism does not appear to be a factor of significance in relation to the presence or absence of hyperglyceridemia, the cause and duration of the hypothyroid state may be relevant, since in our experience the presence of hyperglyceridemia has been limited to subjects with primary spontaneous hypothyroidism wherein the secretion of the thyroid gland may be qualitatively modified as well as diminished in amount. One cannot exclude the factor of time, however, since primary hypothyroidism is often insidious in onset and extant for many years prior to clinical recognition and/or specific therapy.

The presence or absence of hyperglyceridemia is of considerable importance at this time since the hypocholesterolemic effect of androsterone in hypothyroid subjects reported by Hellman et al.[48] has been shown by studies reported from this laboratory[31] to be due mainly to reduction in vld lipoproteins and chylomicrons which follows androsterone administration to euthyroid and hypothyroid subjects alike when these very low density lipids are present in excessive amounts. Androsterone is essentially without effect on serum lipids and lipoproteins, other than for a weak "testosterone-like" action, when the concentrations of vld lipoprotein and chylomicrons are normal.

The serum lipoprotein distribution in hypothyroid subjects in whom hyperglyceridemia as well as hypercholesterolemia is present resembles that noted in hyperglyceridemia of either primary or secondary type (Table XII, subject M.C. compared with O.W. and Table XIII, subject S.M. compared with O.W.).

In comparing hypercholesterolemia secondary to hypothyroidism with primary hypercholesterolemia, one notes that the lipid

XIII
rosis

% of serum value)											
Phospholipid						Cholesterol/Phospholipid Ratio				Remarks	
β		ldβ		vld							
mg.	%	mg.	%	mg.	%	α	β	ldβ	vld		
89.9 ±4.7	37.8 +1.2	7.9 ±0.7	3.4 ±0.3	28.5 ±2.5	12.3 ±0.9	0.45 ±.01	1.33 ±.04	1.28 ±.12	0.68 ±.03	Mean value and standard error for eighteen normal men	
85.8 ±7.1	37.8 ±2.1	7.8 ±1.2	3.7 ±0.7	16.2 ±1.6	7.5 ±0.8	0.45 ±.02	1.43 ±.03	1.28 ±.20	0.60 ±.08	Mean value and standard error for eleven premenopausal women	
										Weight (kg.)	
383	54	36	5	123	18	0.55	1.57	1.29	0.82	66	Nephrotic syndrome five years with relapse one week
220	48	20	4	85	19	0.43	1.38	1.59	0.78	54	Increased prednisone therapy for two weeks
152	46	14	4	55	16	0.44	1.53	1.57	1.18	63	Nephrotic syndrome for six months; improving but persistent albuminuria; mean of three studies
88	38	7	3	28	12	0.43	1.47	1.43	0.96	61	Further improvement three months later; no corticoid medication; no albuminuria
351	63	28	5	60	11	0.41	1.51	1.38	1.04	64	Nephrotic syndrome for three years; prednisone 20 mg./d. for three days
240	50	3	1	79	16	0.42	1.48	1.64	0.96	70	Renal biopsy "membranous nephritis"; increased prednisone therapy for two months
90	35	21	8	58	22	0.45	1.43	1.48	1.09	60	Mild nephrotic syndrome one year; no recent medication; renal biopsy "membranous nephritis"; mean of two studies
71	19	38	10	178	48	0.34	1.01	1.03	1.26	58	Chronic glomerulonephritis with nephrotic syndrome since age five; no recent corticoids; mean of three studies
133	25	68	13	289	54	0.25	1.10	1.11	1.76	60	Serum obtained three months later during exacerbation; no specific medication
56	12	40	8	254	55	0.29	0.80	1.08	1.24	72	Hyperglyceridemia; mean of two studies

content of the α lipoprotein fraction in hypothyroidism, while quite variable, is often less than that noted in primary hypercholesterolemia, while the lipid content of the ldβ and vld fraction is usually greater. This difference is related to the degree of hyperglyceridemia accompanying the hypothyroidism and is most marked when hyperglyceridemia is severe.

The FFA levels are not remarkable in hypothyroidism.

The administration of thyroid or derivatives (H.S., C.J. and M.C.) restored serum lipids and lipoproteins to a more normal pattern. Serum cholesterol and phospholipid levels diminished and the C/P ratio decreased to values within normal limits. This reduction in serum lipid was due mainly to reduction in the lipid content of ldβ and vld fractions and, to a lesser degree, of the β fraction (H.S. and M.C.). Thus the per cent of the serum lipid found in the β fraction increased while the per cent of the serum lipid in the ldβ and vld fractions decreased. Since no change in the lipid content of the α lipoprotein fraction occurred as other serum lipid values fell, the per cent of the serum lipid found in this fraction increased (Table XII).

HYPERLIPIDEMIA SECONDARY TO NEPHROSIS

Increased serum concentrations of cholesterol and phospholipid (with increased C/P ratio) are characteristic of the nephrotic syndrome[49–52] and are noted in a high percentage of patients early in the course of acute glomerular nephritis.[53] Increased serum triglyceride levels and serum lactescence are not uncommon. Barr, Russ and Eder[54] noted absolute and relative decrease in the cholesterol content of Cohn fractions IV + V + VI (α lipoproteins) and increase in fractions I + III (β lipoproteins) in the plasma of twelve nephrotic subjects. Increased concentrations

of S_f 0–12 (β) 12–20 (1dβ), 20–100 and 100–400 (vld) lipoproteins were noted by Gofman et al.[9] in thirteen patients with the nephrotic syndrome (eleven of whom were under seven years of age), and paper electrophoretic studies by Bossak[55] indicated marked increases in neutral fat and β lipoprotein, decreased α_1 and α_2 lipoproteins. Havel, Eder and Bragdon[3] and Baxter, Goodman and Havel[56] noted increase in d < 1.019 g/ml. and/or d> 1.019 < 1.063 g/ml. lipoprotein fractions in nephrotics. In the more severe cases the d < 1.019 g/ml. fraction was increased, the β lipoproteins normal or nearly so, α lipoproteins diminished. Serum FFA levels are within normal limits but an increased proportion of FFA bound to lipoproteins has been observed.[57] Nye and Waterhouse[58] recently reported increased serum lysolecithin and sphingomyelin levels and decreased lecithin levels in sera from nephrotic subjects. This serum abnormality was attributed to an abnormal phospholipid composition of the d > 1.019 < 1.063 g/ml. lipoprotein fraction alone.

Lipid and lipoprotein data derived from studies in five patients with nephrosis are presented in Table XIII. Inspection of these data reveals that when marked hyperglyceridemia is present (subject S.M.) the distribution of cholesterol and phospholipid among the lipoprotein fractions is the same as that noted in other subjects with primary or secondary hyperglyceridemia. The serum lipid and lipoprotein data from subject O.W. with primary hyperglyceridemia (Table IV) are reproduced in Table XIII for comparison with those from subject S.M. with nephrosis. (Note also Table XII, subjects M.C. and O.W.) The similarity of patterns is striking.

In nephrotic subjects with little elevation of serum triglyceride (subject J.F.) or only moderate elevation (subject E.R.) a much smaller percentage of serum cholesterol and phospholipid is found in the vld fraction, although the actual content of lipid in this fraction may be considerable (subject E.R.), and a larger percentage is found in the α lipoprotein fraction than in subjects with hyperglyceridemia (Table XIII). The lipid and lipoprotein patterns return toward normal as the nephrosis subsides. In the absence of marked hyperglyceridemia, the phospholipid and cholesterol content of the α lipoprotein fraction may be increased, although the per cent of the serum lipid found in this fraction is diminished.

The pattern of lipid distribution in the nephrotic subject in the absence of marked hyperglyceridemia resembles that of subjects with primary hypercholesterolemia or hypercholesterolemia without hyperglyceridemia secondary to hypothyroidism. As is the case in hypothyroidism, α lipoprotein lipid tends to be less and vld lipoprotein lipid greater, with increasing serum triglyceride levels. Our studies of the serum lipid and lipoprotein distribution agree well with those of Baxter, Goodman and Havel[56] who suggest that mild to moderate nephrosis is associated with increase in d > 1.019 < 1.063 g/ml. (β) lipoproteins and that, as the syndrome becomes more severe, d < 1.019 g/ml. lipoproteins and triglycerides increase in quantity and lactescence appears. At this stage β lipoproteins diminish to or below normal values. Rosenman, Byers and Friedman[59] have suggested that triglyceride accumulation, due to a disturbance of the lipoprotein lipase mechanism, leads to passive accumulation by "trapping" of cholesterol and phospholipid in the blood. The effect of sonic forces on the lipoprotein distribution in hyperglyceridemic, hypercholesterolemic serum from nephrotic subjects is similar to that noted in serum from subjects with primary hyperglyceridemia.

SUMMARY AND CONCLUSIONS

The serum lipids (total and free cholesterol, phospholipids, free fatty acids and triglycerides) and lipoproteins have been studied in normal subjects of both sexes and in patients with a variety of hyperlipidemic disorders including primary ("essential," familial) hypercholesterolemia, primary hyperglyceridemia ("essential," familial hyperlipemia) and hypercholesterolemia and hyperphospholipidemia (with or without associated hyperglyceridemia) secondary to diabetes, von Gierke's disease, chronic biliary obstruction, hypothyroidism and nephrosis. The major lipoprotein com-

ponents of the serum were separated by preparative ultracentrifugation at solvent densities of 1.006, 1.019, 1.063 and 1.21 g/ml. after which the cholesterol and phospholipid content of these serum lipoprotein fractions was determined.

The distribution of the serum lipids among the four major lipoprotein fractions of the serum is described in subjects with these disorders as well as the response of these lipids and lipoproteins to certain therapeutic measures in several subjects.

The *in vitro* exposure of serum to sonic oscillation is introduced as a potentially useful new tool in the study of the serum lipids.

Healthy men and postmenopausal women have significantly higher triglyceride concentrations and greater amounts of cholesterol and phospholipid in the d < 1.006 g/ml. fraction (very low density β lipoproteins and chylomicrons) than do premenopausal women. The ratio of α to β lipoprotein lipid, although lowest in young men, is not significantly different in one group from another. The degree of esterification of cholesterol in the several lipoproteins is found to increase with increasing density of the lipoprotein.

In hyperglyceridemia great amounts of cholesterol and phospholipid (as much as 90 per cent of the serum values) are found in the d < 1.006 g/ml. lipoprotein fraction, mainly in association with chylomicrons, and the lipid content of the d > 1.063 g/ml. (α lipoprotein) fraction is decreased. Exposure of such serum *in vitro* to sonic forces diminishes the amount of cholesterol and phospholipid in the d < 1.006 fraction and increases the liquid content of the α lipoprotein fraction, suggesting an association of α lipoproteins with very low density β lipoproteins and chylomicrons in this disorder.

The lipoprotein abnormality characterizing hyperglyceridemia is similar in primary and secondary types.

The excess serum cholesterol and phospholipid in primary ("essential") hypercholesterolemia are present in the d > 1.019 < 1.063 g/ml. (β lipoprotein) fraction while the lipid content of the α lipoprotein fraction is usually normal.

The hyperlipidemia associated with hypothyroidism is noted to be of two types: one in which serum triglyceride levels are elevated, the other in which they are normal. In the nonhyperglyceridemic type a "hyperbeta-lipoproteinemia" is noted similar to that seen in primary hypercholesterolemia.

The hypercholesterolemia of the nephrotic state, as is the case in hypothyroidism, may or may not be accompanied by hyperglyceridemia. In the absence of hyperglyceridemia the pattern of the serum lipid distribution resembles that seen in primary hypercholesterolemia or hypercholesterolemia (without hyperglyceridemia) secondary to hypothyroidism.

The serum lipoprotein pattern characterizing hypercholesterolemia with marked hyperglyceridemia secondary to hypothyroidism or nephrosis is similar to that of other primary and secondary hyperglyceridemic states. The lipid content of the α lipoproteins tends to be diminished, while that of the d < 1.006 g/ml. fraction (very low density β lipoproteins and chylomicrons) tends to be increased, to a degree reflecting the magnitude of increase in serum triglyceride levels.

In chronic biliary obstruction there are excessive amounts of cholesterol and phospholipid, especially the latter, in the d > 1.019 < 1.063 g/ml. ("β lipoprotein") fraction and moderate to marked diminution of α lipoprotein lipids. The *in vitro* exposure of serum from subjects with biliary obstruction to sonic forces causes a marked diminution in cholesterol and phospholipid content of these "β lipoproteins" and a comparable increase in the content of these lipids of the d > 1.063 g/ml. ("α lipoprotein") fraction. This suggests an association of high density lipoproteins with the abnormal "β lipoproteins" characterizing the hypercholesterolemia and hyperphospholipidemia of chronic bilary obstruction.

Serum free fatty acid levels do not appear to be abnormal in any of these diseases characterized by abnormal serum lipids and lipoproteins.

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Studies on Enzyme Alterations in the Infantile Sphingolipidoses

Correlation with Pathologic Changes

STANLEY M. ARONSON, M.D.,* ABRAHAM SAIFER, M.S.,† GUTA PERLE, B.S.‡ AND BRUNO W. VOLK, M.D.§

THE FIRST comprehensive description of infantile amaurotic family idiocy (IAFI) firmly established the concept of a genetically oriented disturbance, singularly confined to tissue of the nervous system and characterized by arrested somatic development, cerebral atrophy and early death.[1,2] Subsequent clinical and morphologic contributions have reaffirmed these major representations. Until recent years, the rapid, fatal course of this disorder made it necessary to compress descriptions of the illness into a static profile unassociated with the parameter of time. Prolongation of life, made possible by such adjuncts as improved nursing care and antibiotics, permitted more protracted observations and led to the recognition of a predictable pattern of evolution of disease with assignable clinical,[3] biochemical[4,5] and neuropathologic[6] features at each stage of the disease.

The data in the present report are derived from clinical observations of forty-three patients with verified nervous system sphingolipidoses, many of whom were under continuous biochemical observation over a period of four years. There were thirty-six patients with infantile amaurotic family idiocy, six with Niemann-Pick disease and one with juvenile amaurotic idiocy in this group. The diagnosis of infantile amaurotic family idiocy was based upon the clinical mode of onset, the existence of cherry-red spots in the retinal macula and the absence of any involvement of somatic reticuloendothelial tissues. The thirty cases submitted to necropsy afforded pathologic confirmation of the diagnosis. The six cases of infantile Niemann-Pick disease (NPD) with central nervous system involvement, included in this report for biochemical and pathologic comparisons, were also verified by postmortem examination.

Enzyme activity determinations (aldolase, glutamic oxaloacetic-transaminase, lactic dehydrogenase and phosphohexose isomerase) in serum and cerebrospinal fluid were generally performed at monthly intervals. Only limited data are available on the other enzymes to be discussed (malic acid dehydrogenase, glutathione reductase, leucine aminopeptidase and lipase). Pneumoencephalograms were usually performed at three month periods.

BIOCHEMICAL METHODS

Aldolase in biologic fluids was estimated by

From the Isaac Albert Research Institute, Jewish Chronic Disease Hospital and the Departments of Pathology, State University of New York, Downstate Medical Center, Brooklyn, New York, and the Albert Einstein College of Medicine, Bronx, New York.

* Professor of Pathology, State University of New York, Downstate Medical Center; † Chief, Department of Biochemistry, Isaac Albert Research Institute, Jewish Chronic Disease Hospital; ‡ Research Assistant, Department of Enzyme Chemistry, Isaac Albert Research Institute, Jewish Chronic Disease Hospital; § Director, Isaac Albert Research Institute, Jewish Chronic Disease Hospital and Clinical Associate Professor of Pathology, State University of New York, Downstate Medical Center.

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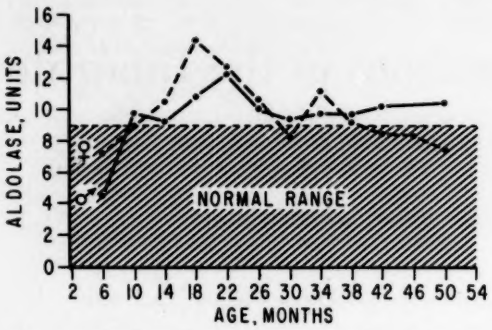

Fig. 1. Serially obtained serum aldolase levels in thirty-six patients with infantile amaurotic family idiocy. A total of 283 separate determinations were available.

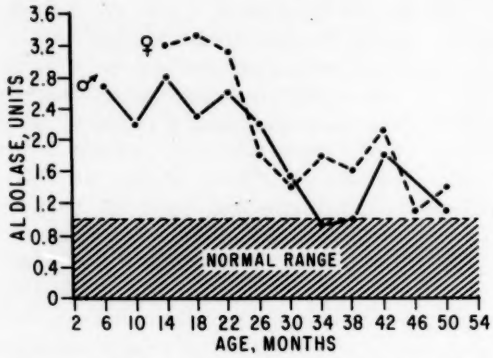

Fig. 2. Serially obtained cerebrospinal fluid aldolase levels (127 separate determinations) in thirty-four patients with infantile amaurotic family idiocy. Note a lack of parallelism between the cumulative curves of Figures 1 and 2.

the colorimetric procedure of Sibley and Lehninger.[7] Glutamic oxaloacetic-transaminase (GOT) levels were obtained through the method described by Karmen, Wroblewski and La Due[8] based on spectrophotometric measurement. Lactic dehydrogenase (LD) activity was also determined spectrophotometrically by the method of Wroblewski and La Due.[9] Bodansky's colorimetric assay for phosphohexose isomerase (PHI) was employed.[10] Leucine aminopeptidase was measured by the colorimetric procedure of Goldbarg and Rutenburg.[11] The method devised by Manso and Wroblewski[12] was used in determining glutathione reductase. Malic dehydrogenase was determined spectrophotometrically according

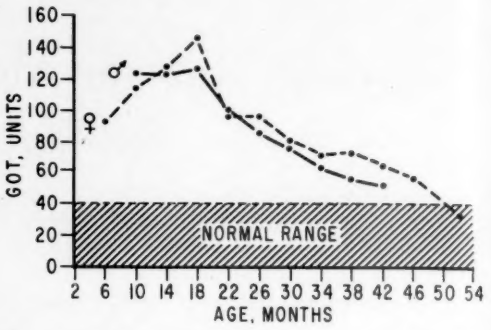

Fig. 3. Multiple serum glutamic oxaloacetic transaminase levels in thirty-five patients with infantile amaurotic family idiocy (269 separate determinations).

to the technic of Siegel and Bing.[13] Technics for the determination of enzyme levels in tissues derived from the nervous system was basically the same as those used for serum and cerebrospinal fluid. Greater care, however, was required to preclude postmortem destruction of enzyme activity in the tissues.

CHARACTERISTICS OF EARLY ASPECTS OF INFANTILE AMAUROTIC FAMILY IDIOCY

This phase or earlier segment of the disorder complies closely to the characteristics classically delineated in previous monographs concerning this illness. There is a certain homogeneity in the features of the illness during the first twelve to sixteen months of detectable disease (up to seventeen to twenty-one months of life) and we believe it is justified to conceive of this portion of the disorder as a distinct phase. The sundry characteristics follow.

Clinical Features. The time of recognition of the first abnormality varied slightly, averaging about five and a half months of life. The primary sign noted by the parents was often an abnormal sensitivity to loud noises (hyperacusis). Shortly thereafter, arrest and regression of development supervened and the child was usually noted to be diffusely paretic, listless and often amaurotic on initial medical examination. Occasionally, abnormal and involuntary limb movements were also noted. Macular degeneration was invariably described. Retardation of growth was also apparent as determined by measurements such as circumference of the chest.

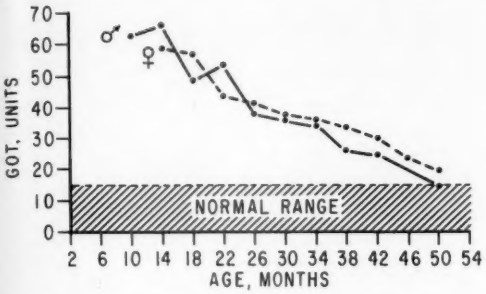

Fig. 4. Cerebrospinal fluid glutamic oxaloacetic transaminase obtained serially in thirty-four patients with infantile amaurotic family idiocy (126 individual determinations).

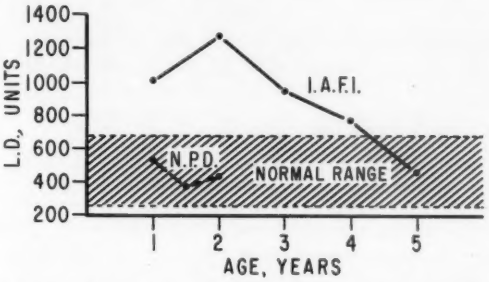

Fig. 5. Serially obtained serum lactic dehydrogenase in twenty patients with infantile amaurotic family idiocy (139 individual determinations) and three patients with Niemann-Pick disease (ten individual determinations).

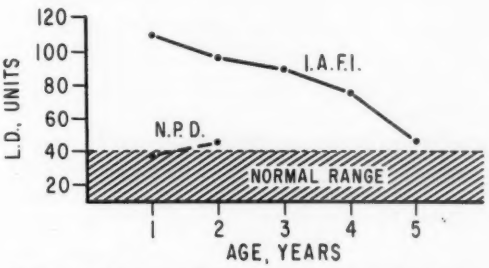

Fig. 6. Serially obtained cerebrospinal fluid lactic dehydrogenase in twenty patients with infantile amaurotic family idiocy (113 individual determinations) and three patients with Niemann-Pick disease (nine separate determinations).

Pneumoencephalography. Exchange of air with cerebrospinal fluid produced a uniformly abnormal radiographic pattern during this phase of the disorder.[3] The internal ventricular cavities were usually dilated. The cerebral tissues were reduced in volume and the air within the supratentorial subarachnoid space was distinctly increased. Obvious gyral atrophy was evident throughout. A comparable degree of infratentorial atrophy was also apparent on radiographic examination and the basilar cisterns were unusually widened.

Serum and Cerebrospinal Fluid Enzyme Studies. The levels of serum aldolase were in the high normal range, rising slightly toward the end of this phase. In contrast, the aldolase activity in cerebrospinal fluid was distinctly abnormal, this change being most apparent in the earliest stages of the disease (Figs. 1 and 2). There was little apparent distinction between the progression of enzyme levels in the male and female infants afflicted.

A dramatic and sustained elevation of serum GOT was characteristic of the initial phase of IAFI. An approximately fivefold increase over normal was apparent throughout this phase, regression of these abnormal values characterizing the protracted phase of the disorder (Fig. 3). The detectable elevation of GOT in the serum may predate the emergence of neurologic symptoms. Abnormally high GOT levels were noted in two children who were otherwise clinically normal. Subsequently, both these infants exhibited the characteristic signs of the disease. The levels of activity of this enzyme in cerebrospinal fluid were also strikingly elevated with a return to normal values as the disease extended to the protracted phase. The highest values were invariably in afflicted children below the age of ten months (Fig. 4).

Serum LD followed a pattern roughly similar to that of serum GOT, although the regression curve was not as precisely correlated in time (Fig. 5). The peak of abnormal values occured during the second year of illness. The values of malic acid dehydrogenase, obtained in a limited number of cases, revealed a close correlation with the deviations exemplified by LD. The LD activity levels in serially studied cerebrospinal fluids exhibited a striking elevation in the initial phases of the disease with a gradual return to normal values in the more protracted aspects of the disturbance (Fig. 6).

The PHI activity levels in both cerebrospinal

TABLE I
Tissue Levels in Infantile Amaurotic Family Idiocy

Region	Control (units/gm. wet tissue)	Phase of Disease		
		1 (0–14 months of age)	2 (15–29 months of age)	3 (over 30 months of age)
A. Glutamic Oxaloacetic Transaminase				
Cerebral gray matter	20,500	14,490	13,200	13,030
Cerebral white matter	16,000	8,330	6,810	7,700
Basal ganglia	20,200	15,600	16,320	12,850
Cerebellum	22,100	16,670	13,460	11,900
Brain stem	27,000	17,840	17,580	14,900
B. Lactic Dehydrogenase				
Cerebral gray matter	78,800	72,640	59,980	65,800
Cerebral white matter	72,000	68,180	42,181	46,150
Basal ganglia	80,000	91,170	88,840	82,500
Cerebellum	85,000	118,530	127,280	137,700
Brain stem	132,000	124,490	131,440	135,000

fluid and serum were in concordance with the determined values of the closely related glycolytic enzyme, aldolase. Serum and cerebrospinal fluid lipase, alkaline phosphatase and leucine aminopeptidase levels were within the range of normal values.

Pathologic Findings. Five of the infants in the present report died during this early phase and all were submitted to autopsy. The central nervous system tissues appeared to be reduced in volume, a change expressed in both supra- and infratentorial tissues, in conformity with the radiographic appearance noted during these earlier months of the disease. The primary cytologic disturbance was centered in the neurons where an intense degree of cytoplasmic storage was evident. Consequently, the argentophilic neurofibrils were compressed and deviated to the periphery of the cell; the Nissl bodies were reduced in number and confined to the perinuclear region. The abnormal storage substance showed positive histochemical reactions with the periodic acid-Schiff (PAS) procedure, were metachromatic with aqueous thionin, very weakly sudanophilic and displayed a positive reaction with Luxol Fast Blue, a copper phthalocyanin reagent believed to react positively with the sphingolipids.[14] The extent of cerebral white matter myelination was less than anticipated but no active demyelination was demonstrable.

Although there was an obvious ubiquity of neurocellular involvement, careful examination of various levels of the neuraxis disclosed that the degree of implication varied considerably in certain areas. The most obvious and most severe ganglionic involvement was in the cortex of the frontal and parietal lobes. Implication of the temporal and occipital cortical neurons was present in a lesser degree. The mildest measure of alteration was in the structures comprising the rhinencephalic system and particularly the small neurons of the olfactory bulb. Similarly, the roof nuclei of the cerebellum and the inferior olivary nuclei were found to be minimally implicated. The quantitative degrees of ganglionic loss among infants with amaurotic family idiocy is as follows:

I. Marked loss
   A. Frontal cortex
   B. Parietal cortex
II. Moderate loss
   A. Temporal and occipital cortex
   B. Basal ganglia, thalamus
   C. Brain stem tegmental nuclei
   D. Spinal cord
III. Mild loss
   A. Rhinencephalon
   B. Olivary and cerebellar nuclei

Consideration of this hierarchy of involvement immediately suggested a parallelism with the phylogenetic pattern of the nervous system. Thus, the more recently evolved structures (e.g., neopallium) were affected most and the more archaic structures were the site of less impressive cytoplasmic storage and consequent cellular destruction.[6] In patients with IAFI surviving for many years such nuclei as the inferior olivary cell mass maintained their architectural configuration and approximate cell population. In contrast, the associative cerebral cortical areas contained only a small residuum of ganglion cells. It is apparent, therefore, that the velocity of the disease proc-

ess varies from one zone of the nervous system to another; there is not a uniformly profound implication previously thought to be characteristic of the disturbance.

Biochemical Studies on Nervous System Tissues. In addition to pathologic studies, two of the brain specimens in this category were also used for certain biochemical procedures. A significant elevation in water content of the tissue and a comparable decline in total protein content was evident in all regions of the nervous system. This was most conspicuous in the cerebral white matter. In general, the depletion of protein content was more evident in the regions of greatest ganglionic implication (cerebral gray matter) and in areas where such ganglionic change was secondarily expressed (cerebral white matter). The tissue content of GOT and LD were also regionally determined. In the values of the former enzyme, a diffuse tissue concentration reduction was revealed which was somewhat more evident in the cerebral white matter. Only an inappreciable reduction of the tissue LD was noted which was considered within the range of normal values. The infratentorial tissues, however, appeared to have an actual increase in tissue concentrations of this enzyme (Table 1). The levels of aldolase in the tissue were also strikingly reduced during the early phase of the diasease.

CHARACTERISTICS OF PROTRACTED PHASE OF INFANTILE AMAUROTIC FAMILY IDIOCY

Clinical Features. Life after thirty months of age was deemed the protracted phase of this disorder. Except for infrequent episodes of convulsive disorders the patient was almost totally immobile. Marked muscular atrophy and inanition were apparent; total blindness was invariable. The limbs were generally areflexic and there was a universal lack of responsiveness to any external stimulation. The retardation in growth continued although, curiously, some of the children displayed a precocious sexual development during this phase of the disorder. Perhaps the most striking alteration in the clinical characteristics of this disease occurred during the protracted phase. This change was a sudden increase in circumference of the head. A precipitous enlargement of the cranial circumference occurred, commencing about the age of twenty-four months. By the age of thirty months an average increase of about 24 per cent was recorded.[15]

Pneumoencephalography. Air studies of the intracranial contents undertaken during the months of the disease coincident with the development of enlargement of the head revealed radiographic alterations which singularly distinguished this phase of the disease. At this time the volume of cerebral tissue was considerably enhanced and the previously determined gyral atrophy and lateral ventricular enlargement had now been replaced by expanded cerebral tissues with secondary ventricular compression. This augmentation was confined to the supratentorial tissues and was not evident in the cerebellum and brain stem. In the latter structures, the atrophy demonstrated radiographically in the antecedent phase of the disease persisted. The basilar cisterns were still dilated.

Serum and Cerebrospinal Fluid Enzyme Studies. The initially high serum levels of aldolase gradually declined to normal limits as the protracted phase of the disease proceded. PHI levels again followed a parallel course. Elevations of aldolase in the cerebrospinal fluid persisted throughout the protracted phase; however, the cumulative data indicated a progressive decline. In rare instances the spinal fluid aldolase activity levels finally approached the upper levels of normal values (Figs. 1 and 2), generally in patients over the age of four years.

The progressive decline in serum GOT levels continued. High normal values were actually noted in two children over the age of fifty months. In no other instances did the declining serum GOT values achieve the normal range. The spinal fluid GOT levels remained elevated with a gradual declination. Patterns of LD and MD were similar.

Pathologic Findings. In contrast to the atrophic shrinkage of the intracranial contents so notable during the initial years of the disease, a considerable megalencephalic expansion characterized the protracted phase of

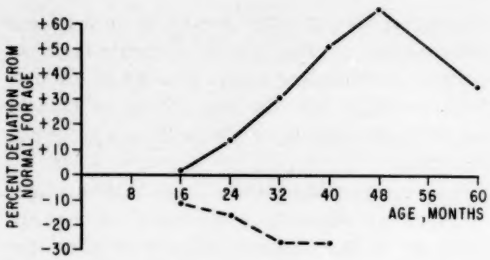

Fig. 7. Brain weights in thirty cases of infantile amaurotic family idiocy and five cases of Niemann-Pick disease with nervous system involvement submitted to complete necropsy. The data are expressed in terms of per cent deviation from normal, equated to age. Closed circles connected by a solid line represent infantile amaurotic family idiocy; closed circles connected by a broken line represent Niemann-Pick disease with central nervous system involvement.

the disease. This volumetric increase, however, was confined to the supratentorial tissues; the cerebellum and brain stem continued to reveal the previously described atrophy. By the age of four years, the average percentage deviation in brain weight had increased by 60 per cent (Fig. 7). The formerly atrophic cerebral white matter had now been replaced by an expanded mass of variably necrotic, intensely edematous, reactive glial tissue. No myelin was demonstrable on gross examination. The lateral ventricles were generally compressed as a result of this expansive change. The cerebral cortex, on the other hand, was reduced in volume and increased in density.

Histologically, the culmination of the disparate ganglionic involvement was now noted. The majority of cerebral cortical neurons (and their axonal extension into white matter) had been destroyed. The predominent cell in the former cerebral white matter was the reactive protoplasmic astrocyte, present in large numbers to the exclusion of almost all other glial components. Frequently these cells were heteromorphic and multinucleated, vaguely simulating neoplastic astrocytes. The quantitative extent of responsive astrocytosis appeared disproportionate to the degree of axonal degradation. In contrast, the gliosis in the cerebellar white matter was quiescent, generally isomorphic and without the cystic degeneration noted in the cerebral hemispheres. Cytologic examination of the reactive astrocytes within the cerebral white matter revealed a number of additional changes. Adherent to most of these cells were poorly defined granules measuring from 0.3 to 2μ in diameter. These minimally basophilic bodies appeared to be adherent to the external cell membrane of the astrocytes and were not contained within the glial cytoplasm. These particles were PAS-positive, minimally sudanophilic and displayed a positive staining reaction with Luxol Fast Blue. At times, there was also a metachromatic reaction with thionin. The positive PAS reaction was not abolished by prior incubation of the tissue in diastase. The histochemical reactions suggested that these granules were probably glycolipids and possibly biochemically altered or protein-bound gangliosides. It is to be emphasized again that the granules were more concentrated in areas of greater glial reaction. Such granules were not demonstrable in the infratentorial tissues.

The normally demonstrable LD activity within neuronal cytoplasm was significantly diminished as the dystrophic storage proceeded and was not apparent in the terminal stages of the disorder. Although LD activity in the resting astrocyte could be demonstrated the extranuclear concentration of this enzyme appeared to be appreciably increased in the cytoplasm of reactive protoplasmic astrocytes.

Biochemical Studies on Nervous System Tissue. A profound depression of tissue GOT and aldolase content characterized the protracted phase of IAFI. A similar reduction was seen in the tissue levels of LD within supratentorial tissues; in contrast, the enzyme level within cerebellum and brain stem appeared to be appreciably increased (Table 1).

NIEMANN-PICK DISEASE WITH INVOLVEMENT OF THE CENTRAL NERVOUS SYSTEM

Studies comparable to those previously described were made in a limited number of infants with Niemann-Pick disease (NPD). While macular degeneration, hyperacusis and many of the other salient clinical features of IAFI were apparent in the six infants with NPD, in no instance was there a clinically

evident megalencephaly. Moreover, when autopsy was performed, the brain weights in each instance was significantly below normal (Fig. 7).[16] Dissection of such specimens usually disclosed diffuse atrophy of all tissues and a complete absence of the deep cystic degeneration so characteristic of protracted IAFI. The serum and cerebrospinal fluid levels of aldolase and GOT were estimated in a limited number of cases of NPD and elevations equivalent to those of IAFI were noted. In contrast, the elevated dehydrogenases (LD and MD) noted in the spinal fluid and sera of infants with IAFI were never noted in those patients with NPD.

COMMENTS

The summarized information, when considered sequentially, indicates that a convenient characterization can be made of the evolution of IAFI. The onset of the disease is variable within narrow limits and it cannot be construed that there is freedom from the disturbance during the early asymptomatic period. Not only are symptoms recorded in numerous cases shortly after birth but also the elevated enzyme levels which preceded symptoms in two of the cases presently reported indicate that the disturbance exists in neonatal and, possibly, in prenatal life.

The early implication of neurons probably causes the retarded maturation of the nervous system. Myelination is partially inhibited and the brain of an infant with IAFI at the beginning of the illness weighs less than is usual. The elevated levels of some of the glycolytic and transaminating enzymes in the biologic fluids are closely associated with the parallel depression of these substances in the affected tissues, thus suggesting that the rise in enzymatic activity reflects a nonspecific liberation from selectively implicated tissues, rather than an abnormally increased intracellular biosynthesis. In every patient with IAFI serially studied the highest levels in spinal fluid were noted at the inauguration of the illness. It must be assumed that the efflux of certain enzymes precedes neurocytolysis, as the peak of actual ganglionic destruction (as opposed to ganglionic enlargement) is not attained until later in the development of the disease.

A close concordancy between spinal fluid and serum GOT is evident. Since no somatic abnormalities have been established in IAFI, it may be presumed that the hypertransaminasemia is the consequence of passage of enzymes through the hemoencephalic barrier.

In the case of aldolase, a divergency between serum and spinal fluid levels is noted. The highest spinal fluid values are obtained in the initial months of the disease when the serum levels are within normal limits. It is conceivable that a different passage threshold between spinal fluid and blood exists, although there is no information in support of this. A hyperaldolasemia does emerge in the second and third years of the disease coincident with the development of severe atrophy of skeletal muscle. Content of aldolase and PHI is higher in muscle tissue than in most other tissues and serum elevation of these enzymes has been equated with numerous primary and secondary myopathic disturbances in the past; therefore, it is reasonable to propose that the serum glycolytic enzyme abnormalities in IAFI are a reflection of the nonspecific atrophy of peripheral musculature.[17,18]

After approximately four years of the disease state the continuous cellular insufficiency and destruction have sufficiently exhausted the source of the elevated biologic fluid levels and, terminally, there is a reversion to the normal range.

Histologic studies have revealed that the cerebral cortical neurons are differentially and most severely affected in IAFI and that a massive neurocytolysis commences during the second year of illness, coincident with the clinical and radiographic evidence of megalencephaly. The cytoplasmic storage substance, composed largely of gangliosides, is released into the interstices and is partially disposed of through microglial scavenger activity. This phagocytic mechanism seems to suffice in neural areas where the neurocytolytic rate is not appreciable (e.g., cerebellum).

In the cerebrum, however, the massive and rapid death of neurons results in a secondary degeneration of the axonal prolongations and

myelin sheaths within the subjacent white matter, ultimately oversaturating the reactive microglial capacity of the supratentorial tissues. As a consequence, large amounts of protein-bound glycolipids presumably derived from cytoplasmic and axoplasmic ganglioside remain in the interstitium as extracellular granules. Concurrently, an astrocytic proliferation supervenes, partially provoked by the overwhelming degradation of cerebral white matter tissue and release of myelin. The astrocytic cell membrane adsorption of glycolipid granules further suggests that these metabolically variant masses may also contribute to the stimulation of astrocytic cell reaction. Certainly, there is no disease of the nervous system which is marked by an astrocytosis of this magnitude and no disease of infancy other than IAFI with such a profound increase of cerebral volume. The re-expanded white matter, together with incorporated fluids, eventually causes a striking supratentorial megalencephaly. In some instances the increase in brain weight was almost threefold.

A considerable turnover of reactive astrocytes is suggested by the histologic finding of both multinucleated astrocytes and those undergoing necrobiosis. Histochemical preparations have demonstrated that there is a considerable amount of LD activity in the cytoplasm of the metabolically active astrocytes. The increases in both serum and spinal fluid LD reach a peak in the second year of disease. This coincides with the peak of responsive astrocytosis, suggesting that the origin of this increased enzyme in biologic fluids can be attributed to the secondary glial hyperplasia. The reciprocal depression of LD activity in the cerebral white matter seems to reflect the accelerated release of enzyme and heralds the ultimate exhaustion of tissue LD from this region. The neurocytolysis and the reactive astrocytosis are less explosive in NPD and the LD levels of the biologic fluids remain within normal limits.

SUMMARY

It is presumed that genetically determined enzymatic defects form the basis of all the infantile sphingolipidoses. In infantile amaurotic family idiocy the precise confinement of the storage process to one type of cell and the observation that greater acceleration of the dystrophic process occurs in nerve cells of phylogenetically recent development further indicates that the enzymatic abnormality in this disturbance is unitary. The demonstration of several enzymatic variations in serum, cerebrospinal fluid and tissues does not justify the designation of any one of these biochemical abnormalities as the basic anomaly of this disorder. Each of these enzymatic abnormalities can be considered reflections of nonspecific neurocytolysis, secondary muscle atrophy or massive reactive gliosis.

The sustained increase of cerebrospinal fluid aldolase, phosphohexose isomerase and glutamic oxaloacetic-transaminase are, in our experience, unique to infantile amaurotic family idiocy and Niemann-Pick disease. The sphingolipidoses are the only neurologic diseases of infancy characterized by progressive, rapid and irreversible implication of *all* nerve cells. Infantile amaurotic family iodocy can be distinguished from Niemann-Pick disease by the differential elevation of the dehydrogenating enzymes in the former. When the rate of neurocytolysis is even more indolent, as in juvenile amaurotic idiocy, no enzymatic elevations can be demonstrated.

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New Methods for the Separation and Quantitative Isolation of Lipids

Initial Applications to the Study of Beef Brain and Spleen Lipids in Gaucher's Disease

GEORGE ROUSER, PH.D.,* A. J. BAUMAN, B.S.† AND GENE KRITCHEVSKY, PH.D.‡

A REVIEW OF THE literature on the sphingolipid disorders makes it apparent that we do not know whether the disorders are of sphingosine metabolism, fatty acid metabolism, or defects related to the metabolism of other parts of the lipid molecules. Certain types of lipids have been found to be present in increased amounts in the tissues of some affected persons. The defect could be related to a decrease in the amount or absence of an enzyme involved in the synthesis or degradation of the storage product, or a more complex substance of unknown nature. It would thus appear that some of the major features of these disorders remain to be discovered. It is for this reason we believe that a thorough investigation of the lipid composition of tissues with strictly quantitative techniques is in order.

What type of approach should we take, what can we expect to find, and what methods are available for such a study? It is our belief that methods for the quantitative isolation and complete characterization of each lipid class must be available before we can understand the functions of lipids in biologic systems in general and the nature of the pathologic processes in the lipoid storage diseases in particular. Characterization must include the determination of the precise fatty acid and fatty aldehyde composition of the lipids that contain these substances.

What can we hope to learn about the lipoid storage diseases following such a complete study? First, by quantitative isolation of each of the sphingolipids in the various disorders we can determine whether or not sphingosine and/or dihydrosphingosine are found as in normal tissues, we can decide whether or not sphingosine biosynthesis is normal. On the other hand, the fatty acid composition not only of the major lipid or lipids deposited but also of all of the lipid classes present may allow us to determine whether or not fatty acid metabolism is normal in these conditions. By a thorough study of the other parts of the lipid molecules, any defects in these areas should be disclosed and new products may be encountered. We are unaware of any report in which quantitative isolation of the major storage products or other lipids have been undertaken in connection with the sphingolipid disorders. The methods used prior to the advent of chromatography have proved to be inadequate for such studies, and the conclusions drawn from earlier studies must be viewed with caution.

Our investigation of the sphingolipid disorders has been divided of necessity into two major phases: the study of methods, and the application of these methods to the investigation of normal human tissues and their counterparts in various pathologic states. As the methods for the quantitative isolation and

From the Department of Biochemistry, Medical Research Institute, City of Hope Medical Center, Duarte, California.

* Senior Research Chemist; † Research Assistant; ‡ Research Associate.

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characterization of most of the nonphospholipids have proved to be more satisfactory than those for the quantitative isolation of phospholipids, we have paid particular attention to the development of methods for phospholipids.

When we began our work on the separation and quantitative recovery of phospholipid classes, aluminum oxide and silicic acid column chromatographic methods[1-3] were available. Although these methods were great improvements over methods previously described, they have proved suitable for the quantitative isolation and characterization of only a few phospholipids from some sources. Phosphatidyl ethanolamine and phosphatidyl serine have been separated only slightly, at best, lecithin and sphingomyelin have proved to be difficult to separate completely, and the lysophospholipids tend to overlap with other compounds when the usual techniques were employed. We have become aware of other inadequacies of these column procedures as we have studied new methods.

The present report is a review of our progress in the development of new methods with some initial applications.

METHODS AND MATERIALS

Quantitative Extraction of Lipids

The first step in any procedure for the quantitative isolation of lipids is quantitative extraction. For this purpose we chose an exhaustive extraction with a mixture of chloroform/methanol, 2/1 (vol./vol.), as recommended by Folch et al.[4] Our procedure is similar to that recommended by Folch except that the extraction is performed under nitrogen, and proportionately more solvent is used.

The fresh, wet tissue was extracted with 10 ml. of solvent per gm. wet weight of tissue by homogenizing for three to five minutes in a Waring blender. Prior to homogenization the solvent had been deoxygenated by passing oxygen-free (prepurified) nitrogen through the mixture. The atmosphere above the solvent in the blender was kept free of oxygen as well. (If the tissue had to be transported, it was quickly frozen in dry ice and, prior to extraction, was chiseled into small pieces.) After the initial extraction, the insoluble residue was filtered off on a sintered glass filter under nitrogen and re-extracted twice with the same volume of solvent used for the first extraction. The pooled chloroform/methanol extracts were evaporated to dryness under reduced pressure and a stream of oxygen-free nitrogen with the temperature being below 10°c. The crude lipid extracts have been dried thoroughly in a vacuum desiccator over potassium hydroxide and mixed thoroughly in a mortar by repeated grinding.

The crude lipid mixture has served as the starting material for our chromatographic studies. No attempt was made to remove water-soluble substances or to separate compounds into classes by solvent precipitation techniques as these procedures may bring about the loss of some lipid. Any loss of material is of particular significance when the fatty acid composition of the lipids is to be examined since the material that is lost may not have the same fatty acid composition as that of the original mixture. Fortunately, such crude lipid extracts have not been found to give rise to particular difficulties.

The necessity for the extraction under nitrogen to prevent oxidative change varies somewhat, depending upon the tissue involved. We have obtained essentially the same results with beef brain lipid whether or not extraction was performed in the absence of oxygen. At the other extreme, however, attempts to perform the extraction of lipid from rabbit appendix in the presence of air have been highly unsuccessful. During homogenization of this tissue with chloroform/methanol, marked oxidative changes have been observed to take place with gross alterations in phosphatidyl ethanolamine in particular. Evidence of alteration of amino phospholipids included the formation of a precipitate in the original extract on standing, the finding of new spots on paper chromatograms and the observation of new peaks on column chromatograms. The formation of these artifacts was prevented by performing the extraction under nitrogen. These findings are in keeping with our previous results.[5]

Just as extraction in air may give rise to difficulties with certain tissues, the failure to store crude lipid mixtures under nitrogen free of

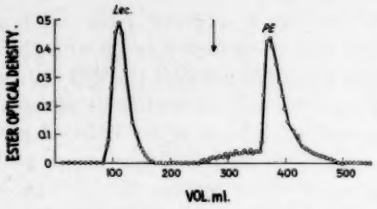

FIG. 1. Separation of soybean lecithin (Lec.) and phosphatidyl ethanolamine (PE) on DEAE cellulose. After elution of Lec. with chloroform/methanol (7/1), PE was eluted with chloroform/methanol (3/2). Solvent change indicated by arrow. Optical density of the ester hydroxamic acid determination on each tube plotted as ordinate.

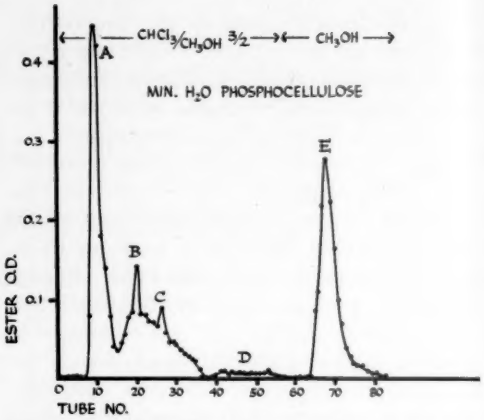

FIG. 2. A = lecithin plus neutral lipids; B and C = peaks from neutral lipid that emerged with phosphatidyl ethanolamine; D = an unknown neutral lipid; E = phosphatidyl serine. Ten ml. fractions were collected. (Ester O.D. = ester optical density in this figure and in Figure 3.)

oxygen may give rise to marked changes in the lipid mixtures. The extent of these changes varies with the source of lipid, but some change has been observed with every crude lipid mixture examined when the preparations have been allowed to stand in an ordinary desiccator that has not been thoroughly deoxygenated with pure nitrogen. It should also be noted that it is necessary to perform column chromatography of phospholipids under nitrogen, particularly when the fatty acid composition is to be analyzed. Both the column material and the solvents should be thoroughly deoxygenated, and the pure fractions recovered must be carefully protected from oxidative change. This is particularly true for the amino phospholipids.

Separation of Phospholipids on Hydrated Silicic Acid-Silicate Columns

Our initial efforts were directed toward the complete separation and quantitative isolation of phosphatidyl ethanolamine and phosphatidyl serine. We have devised several procedures for this purpose. One of the best procedures involves the use of a silicic acid-silicate-water column. Such columns were prepared by passing a mixture of chloroform/methanol/aqueous ammonia through a column of silicic acid. Phosphatidyl ethanolamine was eluted with chloroform/methanol (4/1); subsequently, phosphatidyl serine was eluted with methanol. This procedure appeared to give complete separation and quantitative recovery of these two phospholipids from crude beef brain lipid. The ratio of phosphatidyl ethanolamine to phosphatidyl serine in beef brain was found to be 2.62; it was found that phosphatidyl ethanolamine represents 2.54 per cent, and phosphatidyl serine represents 0.97 per cent of the fresh (wet) weight of beef brain.[6]

Use of Ion Exchange Cellulose Columns

We have demonstrated for the first time that the modified ion exchange celluloses may be used successfully for the column chromatographic separation of phospholipids. Diethylaminoethyl cellulose, commonly referred to as DEAE, was found to be very useful for phospholipid separations. We were able to recover quantitatively and in pure form lysolecithin, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, and some as yet unidentified constitutents from DEAE columns by elution with neutral solvents (chloroform, methanol and mixtures of these solvents). In addition, acidic phospholipids were eluted with mixtures of chloroform/methanol/aqueous ammonia. The elution of lecithin and phosphatidyl ethanolamine is shown in Figure 1. For this run, a mixture of soybean phospholipids was applied to the column, lecithin was eluted with chloroform/methanol, 7/1, and phosphatidyl ethanolamine was eluted with chloroform/methanol, 3/2.

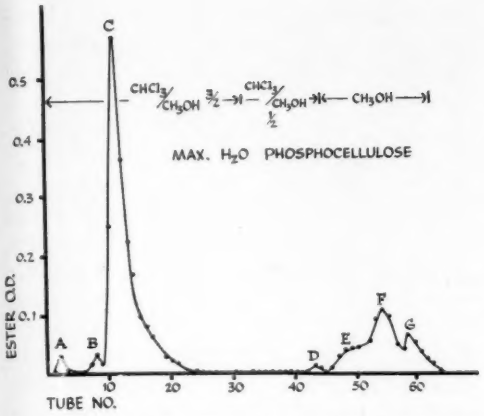

FIG. 3. A and B = neutral lipid peaks; C = lecithin contaminated with neutral lipid; D, E, F and G = peaks of phosphatidyl ethanolamine. Phosphatidyl serine was not eluted in contrast to minimum water content run (Fig. 2). Ten ml. fractions and plot as in Figure 2.

An interesting property of the DEAE cellulose is its ability to bind oxidation products of phospholipids. This is in contrast to the behavior of silicic acid. When a phospholipid mixture had undergone oxidative change prior to application to a silicic acid column, we have observed that some oxidation products of phosphatidyl ethanolamine emerged with or near the parent unchanged compound. That these were oxidation artifacts was indicated by greater absorption at 235 and 275 mμ[7] and by the absence of these substances from unoxidized samples. In contrast to this behavior, such products were held tenaciously by DEAE and were not eluted by chloroform, chloroform/methanol mixtures, pure methanol, or a mixture of chloroform/methanol/aqueous ammonia. The products were eluted with glacial acetic acid. The products eluted from DEAE with glacial acetic acid migrated on silicic acid paper chromatograms in two positions. The first position was that of phosphatidyl ethanolamine and the second approximately that of lysophosphatidyl ethanolamine. Evidently, this behavior of oxidation products could lead to erroneous interpretations if silicic acid chromatography alone were used. These observations should make possible the study of oxidation products of phospholipids present in tissues, where these do in fact exist. It is interesting to note that we have not detected any appreciable amount of material in glacial acetic acid eluates in samples from beef brain that have been processed with care, as described.

We have demonstrated also that phosphocellulose in the sodium, potassium or ammonium form may be used for the separation of phospholipids. In contrast to DEAE, the phosphocellulose columns appear to require water for effective binding of phospholipids. As the water content was increased, more methanol in chloroform was required for the elution of phospholipids. We have been able to elute both zwitterion and acidic phospholipids from this modified cellulose with neutral solvents. Figure 2 shows a typical run with phosphocellulose containing a minimum quantity of water (prepared by washing the column prior to chromatography with 10 column vol. of methanol). One of the most interesting things observed in runs of this type was that phosphatidyl serine could be eluted from the column with a minimum volume of solvent.

In contrast to results with low water content, phosphocellulose of high water content gave the results indicated in Figure 3. In a run of this sort, phosphatidyl serine was difficult to elute and phosphatidyl ethanolamine appeared in the fractions where phosphatidyl serine would have been expected on a column containing a small amount of water. There was evidence also of fractionation of the mixture of phosphatidyl ethanolamines (peaks D, E, F and G). This is presumably upon the basis of differences in fatty acid and/of fatty aldehyde content. It is of interest that carboxymethyl cellulose in the salt form, unlike phosphocellulose, did not retain phospholipids, and that the addition of water to the system did not cause the phospholipids to be bound.

Examination of Fractions Obtained by Column Chromatography

The new methods described were the result of over 150 column chromatographic runs. They could not have been devised had we not used very rapid methods for testing fractions as they emerged from columns, and very rapid paper chromatographic procedures for the

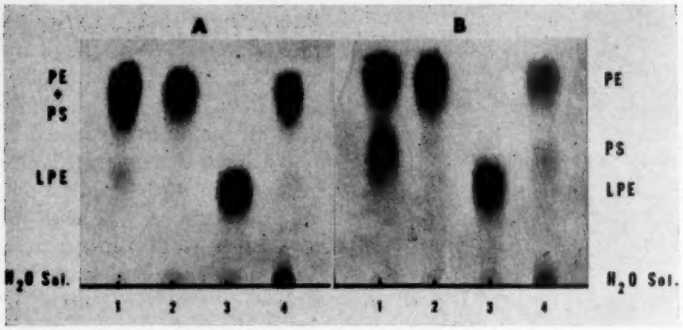

FIG. 4. Illustrated is the effect of water wash on separation of phosphatidyl ethanolamine (PE) and phosphatidyl serine (PS) on silicic acid papers. A, pH of water wash 2.5; B, pH of water wash 5.6. Along the baseline, 1 = 150 μg. crude beef brain lipid; 2 = 150 μg. soybean lipid (no PS); 3 = 40 μg. lysophosphatidyl ethanolamine (LPE); and 4 = 150 μg. crude lipid from rabbit appendix. Silicic acid impregnated paper was developed in chloroform/methanol (4/1) and sprayed with ninhydrin. Note that the migration of LPE and PE was not changed. PS migrated with PE in A and behind PE in B.

characterization of the substances in the fractions. A simple and effective means of following the elution of lipids from a column was to observe the amount of solid material obtained after rapid evaporation of an aliquot in a very hot sand bath. This was a surprisingly sensitive method for lipids that were white powders and the sensitivity for clear, colorless lipids was improved by the addition of sulfuric acid after evaporation of the solvent, followed by heating to obtain charring. The sensitivity was, of course, increased by taking large aliquots of those fractions that contained small amounts of lipid. With the volatile solvents used for chromatography, evaporation of a fraction was completed in a large test tube before the next fraction emerged from the column.

We have used a very simple and rapid ninhydrin test that made possible the detection of ninhydrin positive constituents as they emerged from a column. The test was conducted by the addition of 0.1 ml. of a fraction to a mixture of 0.1 ml. lutidine (or pyridine) and 0.1 ml. of ninhydrin reagent (1 mg. ninhydrin per ml. of *n*-butanol). The mixture was heated in a very hot sand bath (over 200°C.) in a tapered 1 or 2 ml. centrifuge tube. Color development was observed in from thirty to sixty seconds, and by looking down into the tube held against a white background minute traces of amino phospholipids were detectable. We found that evaporation of large volumes of column effluent failed to disclose a ninhydrin positive residue when this test was negative for traces of material.

Two types of chromatographic systems were employed for the characterization of the lipid materials in individual column fractions. Paper chromatography of the original fraction was carried out and followed by an examination of the characteristic hydrolysis products of the phospholipids with equally sensitive methods. It is important that each substance be studied by several methods since unidentified lipids may be present in tissues and artifacts may appear.

Paper Chromatography of Phospholipids

The paper chromatographic method described by Lea, Rhodes and Stoll,[2] using silicic acid impregnated paper and chloroform/methanol (4/1) as the solvent, has several drawbacks. Phosphatidyl ethanolamine and phosphatidyl serine are not separated in this system, and the clear recognition of inositol phospholipids and lysophospholipids has not been possible in every case. This type of silicic acid paper is sufficiently acidic to bring about partial decomposition of the plasmalogen form of phosphatidyl ethanolamine during the application of the pure substance to the paper. Because of these difficulties, we developed a new

rapid procedure using silicic acid-silicate impregnated filter papers and a mixture of chloroform/methanol (4/1), containing 5 to 10 ml. of concentrated aqueous ammonia per liter, as developing solvent. The development time was as short as one to two hours.

A carefully standardized method for preparing silicic acid-silicate paper was used. Whatman No. 3 or 3MM paper was washed by capillary descent, first with 2N acetic acid and then with 95 per cent ethanol.[8] The dry paper was then cut into strips 7 by 9 or 7 by 18 inches and dipped into sodium silicate solution (Mallinckrodt sodium silicate, 40° to 42° Be, diluted with an equal volume of distilled water). After the papers were wet with sodium silicate, they were dipped into a solution of 6N hydrochloric acid saturated with sodium chloride (for every ten sheets of paper 7 by 9 inches, 250 ml. of acid was used). The ten sheets were placed in acid in order, and removed in the same order after the last sheet had been added. The papers were then transferred to 2 L. of distilled water for an initial wash in a glass tray. The papers were then transferred to another tray containing 2 L. of distilled water and the process was repeated for a total of four washes. The pH of the final wash water was about 5.0 to give silicic acid-silicate paper that gives separations similar to those obtained on hydrated silicic acid-silicate columns. The silicic acid-silicate paper may be developed with a mixture of chloroform/methanol (4/1) however, the same mixture containing aqueous ammonia has proved to be superior as phosphatidyl ethanolamine and phosphatidyl serine are more widely separated, and inositol phospholipid migrates well behind lecithin. In addition, lecithin and sphingomyelin were quite widely separated when the ammonia containing solvent was used.

The addition of sodium chloride to the hydrochloric acid for the preparation of impregnated paper was adopted after it was observed that some sodium silicate solutions yielded a silicic acid that was rather soluble in acid alone. Figure 4 illustrates the importance of the standardization of the wash with distilled water. For this study papers were removed from a batch after different degrees of

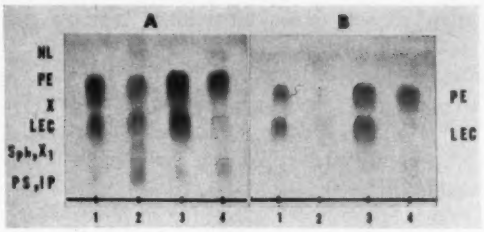

FIG. 5. Use of *p*-rosanilin stain. A, freshly prepared, nonspecific reagent. B, reagent allowed to stand for a few minutes in an open tray has become almost specific for plasmalogens. Along the baseline, 1 = 150 μg. crude beef heart mitochondrial lipid; 2 = 150 μg. soybean phospholipid; 3 = same as 1, but 300 μg. of the lipid, and; 4 = 150 μg. crude beef brain lipid. Phosphatidyl ethanolamine (PE), X (phosphatidyl glycerol ?), lecithin (LEC), sphingomyelin (Sph), phosphatidyl serine (PS), inositol phosphatide (IP) and an unidentified substance (x_1) in soybean lipid all gave the nonspecific test. Only PE in brain lipid and PE and LEC in mitochondrial lipid showed appreciable amounts of plasmalogen. Chromatograms were run on silicic acid-silicate paper and were developed in chloroform/methanol (4/1) containing 10 ml. of concentrated aqueous ammonia and 1 gm. of ammonium acetate per L. The running time was about two hours.

washing and dried. Phospholipid preparations were applied to the paper and the chromatograms were developed in a mixture of chloroform/methanol (4/1). Phosphatidyl ethanolamine and phosphatidyl serine migrated together on the most acidic paper, but as the acidity was reduced a complete separation of these two compounds was obtained. When the chloroform/methanol/ammonia solvent was used, both silicic acid and silicic acid-silicate papers gave similar results; however, as indicated previously, some hydrolysis of plasmalogen may take place during application to the acid papers. If this possibility is not taken into consideration, it may be assumed erroneously that the lysophospholipid formed is present as a contaminant.

We used some highly sensitive and improved reagents for the detection of compounds on paper chromatograms. One of the most important modifications was that of the rhodamine staining technique that we introduced originally.[8] The modified stain was a 0.001 per cent solution of rhodamine 6G in 0.25 M dipotassium hydrogen phosphate. The addition of the potassium phosphate greatly intensified the yellow color of the lipid spots

when viewed under ultraviolet light. When paper chromatograms were dipped for a minute or so in this reagent, rinsed for a few seconds with tap or distilled water to remove excess dye, and viewed under ultraviolet light, approximately 1 μg. of many of the lipids could be detected.

As it is frequently desirable to illustrate results photographically and rhodamine 6G is not suitable for this purpose, we have found the use of a surprisingly sensitive visible stain with *p*-rosanilin (National Aniline) to be of great value. The reagent contained 0.5 gm. of *p*-rosanilin, 8 ml. of concentrated hydrochloric acid and 5 gm. of sodium bisulfite per liter and was decolorized with charcoal before use. The freshly prepared reagent gave blue spots in visible light with most lipids, regardless of whether they contained aldehydes as constituents (Fig. 5A). When this reagent was allowed to stand, it became more specific for the plasmalogen (aldehyde containing) forms of the phospholipids (Fig. 5B).

In our search for ways to improve the sensitivity of the ninhydrin reagent for the location of amino phospholipids on paper chromatograms, we found the following method to give the best results. The thoroughly dried chromatogram was dipped for two minutes in 6 N hydrochloric acid washed for several minutes in running tap water to remove excess acid and dried in an oven at about 100°c. (The entire procedure required less than ten minutes.) The dried papers were then sprayed with the ninhydrin reagent prepared by mixing (immediately before use) 4 volumes of the stock solution of ninhydrin in *n*-butanol (1 mg. per ml.) with 1 volume of lutidine or pyridine. After spraying, the chromatograms were heated at 120°c. for three to five minutes for development of full color. The results with this reagent are illustrated in Figures 4 and 6. Figure 6 also illustrates the use of paper chromatography to follow the elution of phospholipids from columns.

We developed other more rapid and equally useful paper chromatographic methods. These involved the impregnation of Whatman No. 3 or 3 MM filter paper with 0.5 M solutions of various inorganic salts, followed by the development of chromatograms with chloroform/methanol (4/1). Occasionally other chloroform/methanol mixtures (3/1, 2/1) have been used. The running times have ranged from twenty to sixty minutes. One of the best methods involved the use of 0.5 M dipotassium hydrogen phosphate for impregnation of filter paper and chloroform/methanol (4/1) as solvent. This system was useful as the major phospholipids in most mixtures (phosphatidyl ethanolamine, lecithin, sphingomyelin and neutral lipids) migrated to or near the solvent front. This made the identification of some of the other constituents relatively simple. Certain oxidation products of phosphatidyl ethanolamine appeared just behind phosphatidyl ethanolamine and just ahead of lysophosphatidyl ethanolamine, while phosphatidyl serine appeared behind lysophosphatidyl ethanolamine, and an oxidized form of phosphatidyl serine migrated well behind phosphatidyl serine. This system was thus very useful for the detection of oxidation products and lysophosphatidyl ethanolamine, and for confirmation of the identification of phosphatidyl serine. Phosphatidyl inositol was observed to migrate between lysophosphatidyl ethanolamine and phosphatidyl serine.

Sodium molybdate (0.5 M) gave results which were similar to those obtained with sodium or potassium phophates. Sodium sulfate and sodium carbonate (0.5 to 2.0 M) were found to be useful as they bound phospholipids more strongly than phosphate or molybdate salts with the results that neutral lipids migrated to the solvent front and lecithin and sphingomyelin separated just behind the neutral lipids. Phosphatidyl ethanolamine, lysolecithin, lysophosphatidyl ethanolamine, phosphatidyl inositol and phosphatidyl serine migrated more slowly and all were separated. It was observed that a variety of inorganic salts could be used, and even sodium chloride or barium acetate gave separations, although they were not as useful. All of these systems are very fast. Migration down a 9 inch strip of paper requires from twenty minutes to an hour at 25°c.; thus, these systems can be used routinely in testing fractions as they emerge from columns. In general, the various salt

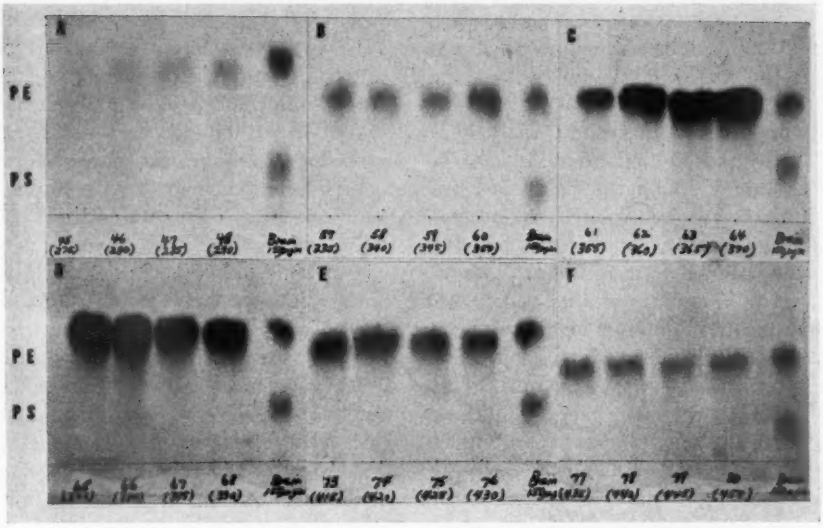

FIG. 6. Fractions from the phosphatidyl ethanolamine (PE) peak recovered from DEAE cellulose (column shown in Fig. 2) are given to illustrate the use of the paper chromatographic system of Figure 5 in column chromatography. Chromatograms (A–F) were sprayed with ninhydrin. 100 μl. of each fraction was spotted on paper and 150 μg. crude beef brain lipid was spotted on each as a control. Note the rise and fall of PE content and the absence of phosphatidyl serine (PS seen in beef brain mixture) in the fractions.

papers gave the same relative order of migration of compounds; however, as phospholipids were retained more tenaciously by some salts than by others, useful differences existed.

Paper Chromatography of Hydrolysis Products

The methods for examination of isolated lipids should include the demonstration of the characteristic hydrolysis products when a new lipid mixture is examined. Any fatty acids released can be determined by gas chromatography of the methyl esters. Ideally, one should demonstrate both the presence of typical water-soluble hydrolysis products and the absence of those products characteristic of other lipids. For example, the characterization of phosphatidyl ethanolamine should include a demonstration of the presence of ethanolamine and glycerol phosphate, and the absence of serine or other amino acids, choline and carbohydrates. Simple paper chromatographic methods are useful for such studies since they are sensitive and rapid.

One method of making the paper chromatography examination extremely sensitive is to select a solvent system in which the hydrolysis products characteristic of the lipid under examination migrate to the solvent front, while contaminants migrate well behind the solvent front. This method was used and described in connection with previous studies.[5] We found that it was possible to apply almost 1 mg. of ethanolamine and detect less than 1 μg. of serine as a contaminant using phenol saturated with water as solvent.[5,6] The complete absence of serine from phosphatidyl ethanolamine isolated from beef brain using a silicic acid-silicate-water column, was demonstrated, but this sensitive method disclosed trace amounts of at least four other ninhydrin positive materials.[6] The nature of these substances is unknown. These traces of what appear to be amino acids may be present in a compound similar to phosphatidyl ethanolamine in having one free amino and one phosphate group, but containing amino acids. On the other hand, these may be artifacts.

Paper Chromatography of Neutral Lipids

The neutral lipids (sterols, sterol esters,

mono-, di- and triglycerides, free fatty acids, coenzyme Q and cerebrosides) are much less polar than the ionic phospholipids, and the bonds linking the neutral lipids to various adsorbents are weaker, in general, than those involved with phospholipid binding. As a result of the weaker binding to adsorbents, less polar solvents are required to elute the neutral lipids from adsorbents. It is thus possible to use silicic acid, silicic acid-silicate, sodium phosphate, sodium sulfate, sodium carbonate, magnesium silicate, aluminum oxide, etc. for impregnation of filter paper, and various mixtures of hydrocarbons with solvents of increasing polarity (benzene, diethyl ether, chloroform and alcohols) for the separation of these substances.

For the separation of cholesterol, cholesterol esters, mono-, di- and triglycerides and coenzyme Q, we found two systems to be particularly convenient. Silicic acid paper was developed with 5 per cent ether in *n*-hexane. The running time of this system was approximately one hour for solvent migration of 6 to 8 inches in a chamber with a solvent-saturated paper liner. This was adequate for the separation of the substances mentioned except that coenzyme Q overlapped slightly with triglyceride. Coenzyme Q was distinguished from triglyceride as it gave an intense purple spot under ultraviolet light. The lipids were localized by dipping in the rhodamine 6G stain described for phospholipids. A rapid system (thirty minutes for 8 to 10 inches of solvent migration in a chamber lined with paper saturated with solvent) was obtained by the use of filter paper impregnated with 2.0 M sodium carbonate. After spotting on the lipid sample, the paper was developed in hexane or 5 per cent ether in *n*-hexane. It was convenient to use pure diethyl ether as the developing solvent for the recognition of monoglycerides. Phospholipids, fatty acids and cerebrosides did not migrate from the origin on carbonate paper.

Paper chromatographic separation of cerebrosides was readily accomplished on magnesium silicate paper. Magnesium silicate paper has been prepared in two ways. Commercial magnesium silicate (Florisil®) was ground to a fine powder in a mortar with a pestle and then rubbed into Whatman No. 3 or 3 MM paper. Alternatively, the paper was dipped in sodium silicate and then treated with magnesium chloride to form magnesium silicate in the paper. Paper prepared by the latter procedure was somewhat more uniform. These papers were developed with chloroform/methanol mixtures. For the separation of beef brain and Gaucher's spleen cerebroside, a mixture of chloroform/methanol (9/1) was satisfactory. For the separation of the somewhat less polar ceramides (such as yeast cerebrin or the ceramide produced from brain cerebroside by hydrolysis) a mixture of chloroform/methanol (35/1) was required. The cerebrosides were localized by staining with the rhodamine 6G reagent. As would be expected, cerebroside sulfate chromatography was accomplished with the systems suitable for phospholipids since this substance is ionic. Again, as would be expected, cerebroside sulfate migrated very much like phosphatidyl inositol.

Initial Studies of Gaucher's Disease

With the development of the new methods just described and the extension of previous methods, we believe that we have passed through the first stage of our studies on the sphingolipid disorders, i.e., the development of more adequate methods for the isolation and characterization of lipids. Other promising techniques are illustrated by our initial studies of tissues from patients with Gaucher's disease in which we used magnesium silicate (Florisil) and silicic acid columns. These studies appear to represent the first attempts at the quantitative recovery of cerebrosides and other lipids from tissue of patients with Gaucher's disease.

Typical Gaucher's cells were observed by Dr. Alfred Knudson in bone marrow smears from a male child (M. F.), the youngest of a family of four children, at the age of seven months. The cells were not observed four months after birth. Early diagnosis was possible as two of the other children in the family had been found to have the same disorder. None of the children in the family showed any signs of involvement of the central nervous system. Surgical removal of the spleen of M. F. was performed at the age of two and a half years be-

TABLE I

Florisil Column Separation of Spleen Lipids Gaucher's Disease

Fraction	Volume (L.)	Solvent	Substance	Weight (gm.)
1	3	Et	ch, chE, coQ	0.304
2	3	C:M (4:1)	cer	1.118
3	3	C:M (4:1)	X_1, X_2,	0.101
4	3	C:M (4:1)	X_1, X_2, PL	0.069
5	3	C:M (4:1)	X_1, X_2, PL	0.055
6	3	C:M (4:1)	X_1, X_2, PL	0.049
7	3	C:M (4:1)	X_1, X_2, PL	0.044
8	3	M	PL, X_2, WS	0.804
9	3	M	PL, X_2, WS	0.245
10	3	M	PL, X_2, WS	0.309
			Total	3.098*

NOTE: cer = cerebroside; C = chloroform; ch = cholesterol; chE = cholesterol esters; coQ = coenzyme Q; Et = diethyl ether; M = methanol; PL = phospholipids; WS = water soluble; X_1, X_2 and X_3 = unknown substances.

* The total weight of substances recovered represents 103 per cent of the 3.00 gm. applied.

TABLE II

Silicic Acid Column Chromatographic Separation of Neutral Lipids

Fraction	Volume (ml.)	Solvent	Substance	Weight (gm.)
1	150	H	P + HC	0.004
2	500	15% B in H	chE	0.019
3	1300	5% Et in H	TG, coQ	0.001
4	1000	15% Et in H	ch	0.215
5	600	30% Et in H	DG	0.004
6	600	50% Et in H	?	0.004
7	600	100% Et	MG	0.002
8	500	M	?	0.003
			Total	0.252*

NOTE: B = benzene; ch = cholesterol; chE = cholesterol esters; coQ = coenzyme Q; Et = diethyl ether; H = n-hexane; HC = hydrocarbons; M = methanol; MG, DG and TG = mono-, di- and triglycerides; P = pigments.

* The total weight of substances recovered represents 87 per cent of the 0.290 gm. applied. There was an approximate loss of 13 per cent coQ.

cause of secondary hypersplenism.

A crude lipid extract weighing 11.5 gm. was prepared (as previously described) from 194 gm. of spleen and used for column chromatography. A Florisil column was used for initial fractionation. The elution of neutral lipids with ether, and cerebrosides with chloroform/methanol (4/1) was carried out as described by Kishimoto and Radin.[9] Additional fractions were collected as shown in Table I and each fraction was examined with the aforementioned paper chromatographic techniques.

Paper chromatography of the ether eluate demonstrated the presence of substances migrating like cholesterol and cholesterol ester. It also showed a compound that overlapped with triglyceride that could be located on chromatograms without staining as a purple spot under ultraviolet light. From the yellow-orange color of the compound in visible light, as well as its chromatographic migration, it was thought that the substance might be coenzyme Q (ubiquinone). Comparison of the chromatographic migration of this compound with an authentic sample of coenzyme Q_{10}, obtained from Dr. Sidney Fleischer, confirmed this expectation as the two substances migrated together and gave the same purple spot under ultraviolet light. Coenzyme Q was distinguished readily from an authentic sample of vitamin K_1 by chromatography.

The ether fraction, eluted from Florisil was then fractionated on silicic acid using the procedure of Barron and Hanahan.[10] The results are shown in Table II. The identification of cholesterol and cholesterol esters was confirmed by infrared and paper chromatographic examination. Cholesterol represented 7.17 per cent and cholesterol ester 0.63 per cent of the crude lipid. Coenzyme Q was lost completely during the run, presumably from the effects of light and air. If the loss of 38 mg. is assigned to coenzyme Q, then the compound would represent 1.26 per cent of the total crude lipid.

The cerebroside fraction (fraction 2 of Table I) represented 37.3 per cent of the total lipid. This figure is slightly high as a small amount of an unidentified substance that appeared primarily in later fractions could be detected by paper chromatography, although the infrared spectrum of the cerebroside (Fig. 7) was almost identical to the spectrum presented by Rosenberg and Chargaff[11] for the cerebroside from the spleen of a patient with Gaucher's disease and to the spectrum of a sample of beef brain cerebroside prepared in this laboratory.

Fractions 3 and 4 contained two unidentified lipids (X_1, X_2) which were clearly distinguishable from cerebrosides, lecithin, phosphatidyl ethanolamine, phosphatidyl serine, phosphatidyl inositol, sphingomyelin and cerebroside sulfate by paper chromatography. Both were ninhydrin negative. Only traces of the

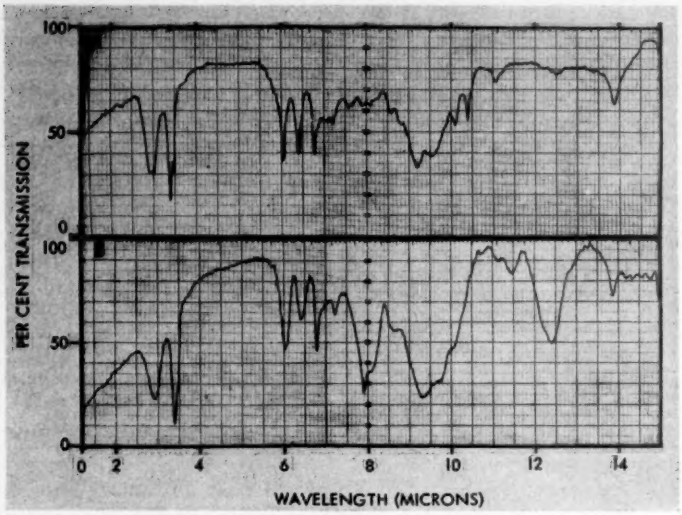

FIG. 7. Infrared spectra of (A) cerebroside from the spleen of M.F. (Gaucher's disease) and (B) cerebroside sulfate (from beef brain). The samples were pressed in potassium bromide.

common phospholipids appeared in fraction 4.

Fractions 5, 6 and 7 contained the new compounds plus increasing amounts of phospholipids. Fraction 8 contained the first portion of a third unidentified compound (X_3) with a small amount of the usual phospholipids. Fractions 9 and 10 contained principally the unidentified substance (X_3) plus water soluble material.

The unknown substances were clearly different from cerebroside or cerebroside sulfate prepared by the method of Lees et al.,[12] when examined in the infrared spectra (Fig. 7).

These studies have disclosed the following: (1) the presence of what appears to be coenzyme Q in the spleen, (2) the large amount of cerebroside that may be deposited, (3) the absence of mono-, di- or triglycerides, and (4) at least three unidentified compounds that appear to make up a significant percentage of the total lipid. The purification and characterization of these new substances may shed new light on the nature of this form of Gaucher's disease.

CONCLUSION

New methods are presented for the isolation and characterization of the lipids, both phospholipids and nonphospholipids, in normal and pathologic human tissues. We can expect to gain a new and deeper insight into the nature of the sphingolipid disorders with these more nearly quantitative procedures coupled with the more accurate techniques for the characterization of lipids.

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Incorporation of P^{32}-Labeled Orthophosphate into Tissue Phospholipids of Intact Animals

Summary

GERHARD SCHMIDT M.D.,* LOUIS H. FINGERMAN, M.D.,† HERBERT M. KREEVOY, B.A.,‡
P. DEMARCO§ AND S. J. THANNHAUSER, M.D., PH.D.‖

THE DIFFERENT phospholipids of a given tissue differ strongly in their respective rates of incorporation of P^{32}-labeled orthophosphate.[1-3] The studies summarized herein yielded information concerning the behavior of the plasmalogens; the phosphorus metabolism of the plasmalogens had not been investigated hitherto owing to the lack of suitable analytic methods.

ANALYTICAL PROCEDURE

A procedure for the determination of the specific radioactivity of P^{32}-labeled plasmalogens was developed on the basis of the selective and quantitative conversion of phosphatidyl compounds, plasmalogens and sphingophospholipids to water-soluble phosphorus compounds as described by Schmidt et al.[4] In this technic, the separation of lipid phosphorus from the water-soluble P-compounds formed during the specific degradation steps is accomplished by precipitation of the former with ammonium sulfate at high concentration, followed by phosphorus analysis of the aqueous filtrates. Owing to their high salt content, such filtrates are not suitable for measurements of radioactivity. The original technic was adapted for application to studies of incorporation by using the purified lipid precipitates rather than the aqueous filtrates for determinating the specific radioactivities.

RESULTS

Representative figures for the relative specific radioactivities of the total phospholipids and of the water-soluble phosphorus compounds of tissues of rats are given in Table I. Each animal was killed three hours after receiving an injection of P^{32}-labeled orthophosphate. Each value for relative specific radioactivity is the quotient between the specific radioactivity of the indicated phosphorus fraction and that of the specific radioactivity of the total phospholipid phosphorus of the liver.

Representative figures for the ratios between the specific radioactivities of the respective individual phospholipids and those of the total phospholipids in some tissues of rabbits are given in Table II. Each rabbit was killed three hours after receiving an injection of P^{32}-labeled orthophosphate.

CONCLUSIONS

In agreement with earlier results of other investigators,[5] it was found that orthophosphate is incorporated into the phospholipids of the heart much faster than into those of the skeletal muscles (Table I). Possibly this difference is a direct consequence of the simi-

From the Boston Dispensary and the Department of Biochemistry, Tufts University School of Medicine, Boston, Massachusetts.

* Professor of Biochemistry; † Lederle Research Fellow; ‡ Research Fellow; § Research Assistant; ‖ Clinical Professor Emeritus of Medicine.

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TABLE I

Relative Values of the Specific Radioactivities of Lipid and Acid Soluble P Fractions of Rat Tissues after Injection of P^{32}-Labeled Orthophosphate

P-Fraction	Relative Specific Radioactivity				
	Liver	Heart (L.V.)	Skeletal Muscle	Brain	Plasma
Total lipid P	1	0.12	0.025	0.008	0.14
Acid-soluble P	3.0	2.6	0.32	0.1	2.6

TABLE II

Ratios Between the Specific Activity (S.A.) Values of Individual Phospholipid Fractions and Total Phospholipids of Rabbit Tissues after Injection of P^{32}-Labeled Orthophosphate*

Tissue	(Total) Lipid-P	Phosphatidyl-P	Plasmalogen-P	Sphingolipid-P
Heart	100	115	13	5
Skeletal muscle	100	112	20	11.6

* S.A. of individual lipid-P fraction:S.A. of total lipid-P × 100.

larly striking difference observed between the rates of P^{32} incorporation into the acid-soluble P-fractions of these two types of tissues[6] rather than an indication of a more rapid metabolic utilization of phospholipids in the heart.

In the heart and skeletal muscles of rats and rabbits, the rates of P^{32} incorporation into the plasmalogens are considerably slower than those of P^{32} incorporation into the phosphatidyl compounds.

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Letter to the Editor

Effect of Dietary Cholesterol on Serum Cholesterol in Man

Dear Sir:

IN THE MAY 1960 issue of *The Journal of Nutrition*, a paper by Beveridge et al. (pp. 61–65) purports to show that modest additions of cholesterol to a synthetic formula diet fed to students for a short period produced significant increases in the concentration of cholesterol in the serum. The subject of the effect, or lack of it, of dietary cholesterol on the blood concentration in man has been extensively studied, both in surveys and in a wide variety of controlled experiments (*cf.*, *J. Nutrition* 59: 39–56, 1956), but from the paper by Beveridge et al. the uninformed reader might conclude that this is a virgin field. Many of the previously reported experiments covered much longer periods than used by Beveridge et al., with the subjects under constant twenty-four hour surveillance, which was not the case in their experiment.

The authors made no attempt to explain the apparent major difference between their study and others which have been reported. Actually, only the conclusions of Beveridge et al. are novel and their data do not warrant them. The real finding is that in all groups of their subjects the rise in serum cholesterol levels was of statistical significance, whether cholesterol was added to the diet or not. Their data indicate that, compared with a fat-free diet, the provision of 30 per cent of calories from a butter oil fraction provoked a rise in the serum cholesterol level during a period of a few days. Even this conclusion could be questioned in the absence of a continuing control group not receiving the butter oil.

However, Beveridge et al. focus on the supposed effect of the added cholesterol. The data indicate that 95 mg. of cholesterol in the daily diet is associated with a rise, in eight days, or 27.6 mg. cholesterol per 100 ml., which is significant by the ordinary statistical test. But a more significant rise was demonstrated by the control group receiving no cholesterol supplement. The same is true of the serum changes observed in the groups receiving 153 and 293 mg. of cholesterol per day. Only when the intake level was 634 mg. daily is there a suggestion that cholesterol intake plays a part in the blood change, but even in that case statistical analysis fails to show a significant effect of the dietary cholesterol.

Compare the nine subjects in the control group with the nine in group 5, who received 634 mg. of cholesterol daily. The cholesterol changes are reported to have been $+25.3 \pm 5.06$ and $+41.9 \pm 6.74$, respectively. Are these different? The difference is 16.6 ± 8.43, the t value is 1.97 and the difference is not significant (p = circa 0.07).

A better case can be made for the idea that daily cholesterol intakes of 1,295 mg. or more may have an effect, but then there is the difficulty that the rise with 4,493 mg. of cholesterol per day is considerably less than that when the supplement is 2,494 mg. per day, so a linear effect cannot be claimed.

The actual finding of Beveridge et al. was that, when the daily formula diet contained an average of 898 mg. of cholesterol, the average concentration of cholesterol in the serum was about 15.9 mg. per 100 ml. higher than when the diet contained an average of only 142 mg. of cholesterol. This serum difference, although trivial, is more than would be expected from the reports of other experiments—not cited by Beveridge et al. It may be concluded that further experiments are needed to determine what, if any, may be the effect on the serum cholesterol level of man from variations in cholesterol intake within the range of natural human diets.

ANCEL KEYS, PH.D.
Director
Laboratory of Physiological Hygiene
University of Minnesota
Minneapolis, Minnesota

ASCN NEWS

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Founded May 1, 1960

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Constitution and By-Laws

ARTICLE I

Name

The name of the Society shall be "The American Society for Clinical Nutrition, Inc."

It shall be the clinical section of the American Institute of Nutrition. All stationery and other printed matter of the American Society for Clinical Nutrition shall carry, in addition to the official name, the subtitle "Clinical Section of the American Institute of Nutrition."

To assure continuing representation, one member of the American Society for Clinical Nutrition shall be seated on the Council of the American Institute of Nutrition by arrangement between the Councils of each society.

ARTICLE II

Objects

The objectives of the Society shall be to encourage undergraduate and graduate education in human nutrition in health and disease, to provide an opportunity for investigators to present and discuss their research in human nutrition, and to provide a journal or journals for the publication of meritorious work on experimental nutrition. It shall be the further aim of the Society to unify the science and practice of nutrition as it relates to clinical medicine.

ARTICLE III

Membership

Section 1. Eligibility. Candidates will be selected for demonstrated excellence in the field of clinical nutrition. There will be no limitation on the number of members and no restriction because of place of birth, residence, sex, race, or creed. Each candidate must be of unimpeachable moral standing in his profession, in which he must have demonstrated excellence. Each candidate must be acceptable to the Membership Committee of the American Institute of Nutrition as well as to that of the American Society for Clinical Nutrition. Upon approval of his application (*Section 9*), he shall become a member of both organizations with privileges of and obligations to both societies.

Section 2. Qualifications for Membership. Primary consideration will be given to doctors of medicine and other scientists actively engaged in research, teaching, or clinical practice who have demonstrated excellence and continuing interest as manifested by the publication in recognized scientific journals of at least five original reports of investigations. At least two of those shall have been published with the candidate as senior or only author. A substantial number of these publications shall deal with human nutritional problems.

Section 3. Charter members. Charter members will be the original founding group. They must meet the qualifications of *Section 2.*

Section 4 Emeritus members. A member in good standing for three or more years who has retired from active work in human nutrition and metabolism shall be eligible for emeritus membership upon written request to the Secretary. An emeritus member will pay no dues or assessments. Publications of the Society shall be available to him at the same cost as for active members. He shall be entitled to attend all scientific meetings and business sessions, but may not vote nor hold office.

Section 5. Honorary members. The Council may elect two individuals annually to honorary membership as a tribute to their contributions to the understanding of human nutrition and metabolism. Such members need not meet the other requirements for membership. They shall have the privileges of emeritus members.

Section 6. Expulsion. Expulsion from membership for reasons other than those outlined in *Section 7* (failure to pay dues) and *8* (failure to attend annual meetings) shall be upon recommendation of two-thirds or more of the members of the Council when ratified by three-quarters of the membership in attendance at the next annual meeting voting by secret ballot.

Section 7. Obligations. A member in good standing must pay dues and assessments upon notification. Failure to meet such payments within six months, without reason acceptable to the Membership Committee, will be cause for automatic termination of membership. Subsequent renewal of membership must be in accordance with regular application procedure.

Section 8. Attendance. Active membership requires attendance at annual meetings of the Society. Absence from three consecutive annual meetings without unusual cause would terminate membership automatically. The adequacy of this cause will be reviewed by the Membership Committee after receipt of a letter of particulars.

Section 9. Procedure for Application for Membership. (1) Nominations for membership in the Society may be forwarded to the Secretary at any time. (2) Nominations must be proposed and seconded by active or emeritus members in good standing. Each member may nominate no more than three candidates in a given year. Officers of the Society and the Membership Committee may not nominate or second nominations. (3) Each nomination must be accompanied by a curriculum vitae of the applicant, a statement of his qualifications, and a list of his publications. (4) Names placed in nomination before January 31 of each year will be received by the Membership Committee, which will review the qualifications of the candidate, according to *Section 2.* Applications approved by a vote of at least two-thirds of this committee will be voted on by active members at the next annual meeting. The Membership Committee of the American Society for Clinical Nutrition will send an identical list of candidates to the comparable committee of the American Institute of Nutrition for review. (5) An affirmative vote of at least three-quarters of the members present at the annual meeting will be necessary for election.

ARTICLE IV

Rights and Obligations of Members

Section 1. Dues. The annual dues of active members shall be fixed from time to time by the Council. Dues shall consist of (1) dues for membership in the American Institute of Nutrition ($2.00), (2) dues for membership in the American Society for Clinical Nutrition ($2.00), (3) dues for the Federation of Societies ($4.00), and (4) subscription to the official publication, THE AMERICAN JOURNAL OF CLINICAL NUTRITION (see Article VII). All members of the American Institute of Nutrition may receive the official organ of the American Society for Clinical Nutrition at the official reduced rate. All members of both societies will receive *Federation Proceedings* and the programs of the American Society for Clinical Nutrition without additional charge.

Section 2. Professional Activities. A member, upon his induction, assumes the obligations of manifesting continuing interest in the objectives of the Society. He must attend a meeting at least once every three years, and display active interest in the scientific endeavors of the Society.

Section 3. Rights. An active member may participate in the business and scientific sessions of the Society, may submit or sponsor one scientific paper for the annual program, may vote at the corporate meetings, and may be eligible for election to office.

Emeritus members and honorary members shall have all the above rights and obligations except those pertaining to voting election to office, and paying dues.

ARTICLE V

Officers

Officers of the Society shall be a President, a Vice-President who is also a President-Elect, and a Secretary-Treasurer. The President shall be elected for one year, and shall not be eligible for re-election; the President-Elect for two years (the first as Vice-President and the second as President), not eligible for re-election; and the Secretary-Treasurer for a term of three years, not eligible for re-election. If possible, a successor shall be selected one year before he assumes office. A Councilman shall be elected each year to serve a term of three years. (Councilmen elected at the charter meeting of the Society shall serve one, two, or three years.) A Nominating Committee of five members shall be elected annually from a panel of ten members proposed by the Council.

ARTICLE VI

Duties and Limitations

Section 1. Officers. The President, President-Elect, and Secretary-Treasurer shall have the customary duties and rights. The Secretary-Treasurer shall arrange for publication of abstract of papers presented at the annual meeting of the Society. He shall maintain a roster of members and collect dues from them.

Section 2. The Council. It shall be the duty of the Council, in the interim between meetings, to supervise the affairs of the Society, to make all arrangements for the annual meeting, to report on the work of the members, to name ten candidates for the Nominating Committee, five of whom will be elected by the members at the annual meeting, to receive and consider all nominations for membership from the Membership Committee, to report on them at the meeting to which they shall be submitted for election, and to serve as the Editorial Committee of THE AMERICAN JOURNAL OF CLINICAL NUTRITION.

Section 3. Liasion with the American Institute of Nutrition. Officers of the American Society for Clinical Nutrition shall confer with those of the American Institute of Nutrition regarding one member (preferably a Councilman) to be seated on the Council of the parent organization.

ARTICLE VII

Journal of the American Society for Clinical Nutrition

Section 1. THE AMERICAN JOURNAL OF CLINICAL NUTRITION shall be the official organ of the American Society for Clinical Nutrition. It will be furnished to members of the American Society for Clinical Nutrition and the American Institute of Nutrition at a rate of $6.00 per year until such a time as Council elects to publish monthly. Then the rate will increase to $8.50 per year. Contractual arrangements will be made by agreement between the publisher and Council. The Editor and Editorial Board shall consist of an Editor-in-Chief and ten members to be selected by joint agreement of the Editor-in-Chief and Council. The Editor-in-Chief shall be appointed by the Council for a term of five years and may be reappointed for one term. Editorial Board members will serve for five years. Two members of the Board will be appointed each year to replace the two who retire. The Council shall assume the duties of the Editorial Committee and shall be empowered to appoint, on advice of the Editor, the Associate Editors and the Editorial Board. The Council shall assume the responsibility of regulating advertising standards and editorial policies of the Journal. The annual subscription cost shall be included in the dues.

ARTICLE VIII

Meetings of the Membership

Section 1. The annual meeting shall be held in association with the annual meetings of the American Society for Clinical Investigation and the American Federation for Clinical Research in 1961. The annual meeting will make provision for at least one-half day for the presentation of reports on current research by members and non-members. The time and place of future meetings shall be determined by Council.

Section 2. All members of the American Institute of Nutrition shall receive the annual program of the American Society for Clinical Nutrition and be invited to attend.

ARTICLE IX

Committees

The President will appoint: (1) a Membership Committee, (2) an Auditing Committee, and (3) any additional *ad hoc* committees necessary to conduct the business of the Society.

ARTICLE X

Amendments to the Constitution

Amendments to the Constitution must be proposed in writing, and signed by five active members. Such proposals will be sent by the Secretary to the members at least 90 days before the annual meeting. An affirmative vote of three-fourths of those present shall carry the amendment.

A quorum shall consist of at least one Council member and not less than 20 active members in good standing.

Richard W. Vilter, M.D., *President*
Robert E. Olson, M.D., *President-Elect*
Robert E. Hodges, M.D., *Secretary-Treasurer*
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Willard A. Krehl, M.D., *Councilman*

Conference Notes

Malnutrition and Food Habits

Summary of a Conference at Cuernavaca, Mexico, September 9–14, 1960.

ANNE BURGESS, M.D.

IN RECENT years, as the control of communicable disease has improved, the significance of malnutrition has emerged more clearly. The need to understand its causes has become urgent; without this understanding there can be no secure foundation for schemes of prevention.

It is now evident that in many countries malnutrition is largely responsible for the high rate of mortality, especially in children one to four years old. The combination of disease and malnutrition, the one precipitating and perpetuating the other, has a devastating effect in this age group.

Fortunately there is also evidence that improvements in nutrition (and in housing and hygiene) are more effective against the mortality of this older group than against the infant mortality. It is reasonable, therefore, to concentrate upon this group much of the resources of money and personnel that are available for public health work in nutrition.

Various forms of malnutrition as well as general undernourishment are found throughout the world. It has been amply demonstrated, however, by surveys carried out by national and international teams that the most serious and widespread deficiency disease found today is protein-calorie malnutrition in young children. This condition, a state of ill-health found where diets are habitually poor in protein while the calorie intake may vary from gross inadequacy to higher levels, is known by different names in many parts of the world. The severe form, incidentally, in which protein is markedly deficient, is now generally referred to as "kwashiorkor," the name given to the syndrome when it was first described some twenty-five years ago by Dr. Cicely Williams.

The public health significance of protein-calorie malnutrition is twofold, direct and indirect. For each child with frank kwashiorkor in a given area, there may be many others less obviously affected but whose growth and development and resistance to the intercurrent diseases of childhood have become impaired. The malnourished child has little resistance to the common infections, which therefore frequently end in death. This probably explains why the case mortality in certain infectious diseases in children may be 100 to 200 times greater in those areas in which malnutrition is prevalent than in those in which it is rare.

Protein-calorie malnutrition is therefore a serious and worldwide public health problem. The specialized agencies of the UN (FAO and WHO with material assistance from UNICEF) have used their unique international facilities to stimulate interest in and spread knowledge about this important nutritional disease.

One activity has been the organization of conferences at which authorities on different aspects of the problem and on related problems, could come together and exchange their knowledge and experience. Two conferences sponsored by the Specialized Agencies and the Josiah Macy Jr. Foundation took place a year or two ago. The first (1953) dealt with kwashiorkor, and the second (1955) with "Human Protein Requirements and their Fulfilment in Practice." A third (sponsored by the Josiah Macy Jr. Foundation and the World Federation for Mental Health, and supported by the International Agencies concerned with malnutrition) was held in Cuernavaca, Mexico, in September 1960. The problems related to the changes in food habits which will have to take place if people are going to make better use of the resources already available, or accept and use a new food, were considered at this meeting.

There is already enough scientific knowledge to bring about considerable improvement in the nutritional health of children in many parts of the world if it could be widely applied. This knowledge cannot, however, be used where it is needed unless the people themselves want to use it, know how to do so, and are prepared to accept the particular and related changes necessary to the establishment of a better nutritional pattern. Unfortunately, there appear to be psychological, sociological and cultural factors which create barriers against rapid changes in food habits, and which are less well understood than the impersonal aspects of nutrition and malnutrition.

At the conference in Cuernavaca on "Malnutrition and Food Habits," nutrition research workers, pediatricians, public health workers interested in nutritional education and members of the Secretariats of the international organizations sought the help of anthropologists, psychiatrists and social psychologists of wide experience, in elucidating the human factors involved in changing food habits.

Case studies of the problem as it is found in parts of Africa, India and Guatemala presented by Dr. R. F. A. Dean (Director, Medical Research Council Infant Malnutrition Unit, Kampala); Dr. K. Someswara Rao (Deputy Director, Indian Council for Medical Research, New Delhi); and Dr. Nevin Scrimshaw (Director, Nutrition Institute for Central America and Panama, Guatemala), showed the wide variation in social, psychological and economic factors which contribute to the prevalence of protein malnutrition, and condition the form and acceptance of preventive measures.

A day spent at Tlaltizapan, a rural center, brought into focus some of the practical difficulties the nutrition worker meets. There (under the direction of Dr. F. Gomez, Director, Hospital Infantil Mexico), a team of doctors, nurses and social workers are carrying out an intensive study of the beliefs, ideas and practices of the people in order to discover the most suitable approaches to the nutritional improvement of the community. Many of the conditions found in this Mexican village were familiar to most of the participants, nutrition workers from very different parts of the world, and provided a common background to the subsequent discussions.

In a review of the processes involved in culture change, and the lessons learned from the past and from the postwar development of cooperation between nutritionists and social scientists, Dr. Margaret Mead (Associate Curator, American Museum of Natural History) added perspective as well as thought-provoking observations from her own experience of different cultures. Dr. A. T. M. Wilson (Special Adviser, Unilever) applied a psychiatrist's knowledge of the universal human needs and equally universal differences to the question of nutritional change, particularly in relation to the apathetic individual apparently resistant to change, and to the ways in which opinion is formed and changed in a community. He also compared the extensive research and organization deemed necessary in the business world when attempting to change food habits through the process of marketing a new product, with the procedures usually adopted by other agencies.

The social psychologist's experience of market research, attitude and opinion surveys and motivational studies was presented by Dr. J. Stoetzel (Professor of Social Psychology, the Sorbonne, Paris) who also discussed the importance, in the acceptance and rejection of any food, of the "image" presented by that food to the individual.

Realization of the immensely complicated nature of the problem of improving nutritional health was reflected in the different disciplines represented at the conference, and by the discussions themselves. Although some nutritional deficiencies, such as goiter or pellagra, are amenable to action on a national basis, the relief of protein deficiency in children depends ultimately on the voluntary cooperation of the individual family.

Government policy, agricultural practices, the economics of the family, the way food is distributed within the family, the beliefs about what the father or the mother or the child should eat, and many other factors have to be taken into account before any nutrition improvement can be planned. The education required to make any plan effective must be equally comprehensive in its approach, and include also a detailed knowledge of the social and human elements which decide the actions of the individual parent in a given community. The conference recognized and discussed the difficulties inherent in acquiring both the knowledge necessary for such planning and the personnel and skills necessary for long continuing stage-by-stage progress, and made some suggestions as to how these difficulties might be met.

Under the chairmanship of Dr. Otto Klineberg (Professor of Social Psychology, Columbia University, New York) each of the forty participants took every possible opportunity to express their particular point of view, and many aspects of the total problem of malnutrition and food habits, not mentioned in this brief report, were discussed. A fuller report will be published by FAO early in 1961, and should be of much interest and use to nutrition workers everywhere.

Reviews of Recent Books

Protein and Amino Acid Nutrition, edited by Anthony A. Albanese. Academic Press, New York, 1959, pp. 604, $16.00.

This volume represents a revision and considerable expansion of the previously published small monograph entitled "Protein and Amino Acid Requirements of Mammals." The stated objective is "a collection in one volume of rather detailed presentations describing the current state of knowledge concerning this aspect of nutrition." There are sixteen chapters contributed by different authors. The objectives of the various authors differ so widely that it is difficult to arrive at a single evaluation of the book as a whole.

Some of the authors have presented chapters of the kind which might be expected, that is, critical and more or less complete reviews of a particular topic. These include "Utilization of D-Amino Acids" by C. P. Berg; "Methods of Measuring the Nutritive Value of Proteins, Protein Hydrolysates, and Amino Acid Mixtures. The Repletion Method" by D. V. Frost; "The Amino Acid Requirements of Animals" by H. J. Almquist; and "Amino Acid Supplementation of Foods and Feeds" by H. R. Rosenberg. The chapter by H. H. Mitchell on "Some Species and Age Differences in Amino Acid Requirements," which is similar if not identical to that published in the previous monograph, is also stimulating and provocative. The chapter by J. B. Allison on "The Efficiency of Utilization of Dietary Proteins" is appropriate and the thesis should be understood by students in the field.

The authors of many of the other chapters have taken undue advantage of their opportunity. In some of these, the author's own work is republished extensively with long tables, charts and methodology. The extent to which these chapters are germane to the subject matter of the book, worthy of inclusion, or related to the pertinent literature varies greatly. Some of these, like the chapter on "Food Energy and Metabolism of Protein" by P. Swanson, was of interest to this reviewer although she has relied heavily on her own studies. The chapter on "Amino Acid Requirement of Young Adults" by Ruth Leverton is limited to studies on young women in several laboratories in the United States and is a recompilation rather than a re-evaluation. The bias in some of the other chapters is more pernicious in that it may not be recognized unless the reader is well acquainted with the literature and it has been achieved both by the material included and by deliberate omission. For example, the extensive studies by Holt and Snyderman on amino acid requirements of children is only casually mentioned in the chapter on "Protein and Amino Acid Requirements of Children" by A. A. Albanese. This is not justified regardless of the author's appraisal of this work. Some other examples of work which one might expect to find reviewed would be Rose's studies on amino acid requirements of adults or Eagle's studies on tissue culture.

There is a need for a book covering this area of nutrition but this volume does not fulfill the need.

D. M. HEGSTED

Manuel of Applied Nutrition of the Johns Hopkins Hospital, by Janette Carlsen. The Johns Hopkins Press, Baltimore, 1959, pp. 134, $3.00.

This is the fourth edition of a small pocket-size manual of diets as used at the Johns Hopkins Hospital. A wide variety of menu plans are presented, including the "Dental Soft Diet." It was compiled for the staff of that institution and, no doubt, is extremely useful to the members. However, only certain portions, albeit the majority, will be equally useful to others. Various "routines" of local use are described. Any dietitian, however, will be able to develop new ideas from the wealth of material.

One thing is clear in reading this handy book, there is an acute need for standardization of dietetic nomenclature. What is a "limited soft" diet? Should a "convalescent ulcer" regimen contain 4,350 calories? It is hoped that some day various experts will agree among themselves and eventually all hospitals will be talking the same language. S. O. W.

Vitamin B_{12} Metabolism. Some Studies on the Absorption, Excretion, Enterohepatic Circulation, Turnover Rate, Body Distribution and Tissue-Binding of B_{12}, by Peter G. Reizenstein. Gummessons Boktryckeri, Stockholm, 1959, pp. 31.

This work is based on a discussion of six papers by Reizenstein and his colleagues. These studies suggest that the minimum vitamin B_{12} requirement in healthy control subjects is 1.3 or 7 μg. per day. The average requirement in patients with pernicious anemia is probably closer to 7 μg. because of indications of more rapid turnover and impaired enterohepatic circulation. A requirement of 5 μg. per day was taken as a tentative basis for discussing treatment. In healthy subjects the minimum dose of parenterally administered vitamin B_{12} leading to the retention of about 50 μg. seems to be around 200 μg. A requirement of 5 μg. per day was taken as a tentative basis for discussing treatment. In order to enable the body to retain 150 μg. per month, about three 200 μg. injections are necessary.

Adequate parenteral therapy may demand too many injections to be convenient for the patient. Effective

oral treatment combined with the parenteral administration of vitamin B_{12} might be a convenient way of more adequate therapy. O. M. HELMER

Diabetes, edited by R. H. Williams. Paul B. Hoeber, Inc., New York, 1960, pp. 793, $20.00.

Few disorders have been studied more intensively than diabetes; yet each year brings significant new developments so that the literature on diabetes and carbohydrate metabolism is truly voluminous. There exists no paucity of books on the subject. However, this text by fifty-four authors, and well edited by Robert H. Williams of Seattle, is among the very best. No book can be better than its authors, and in this textbook one will find most of the outstanding men in the field who write from a vast personal experience.

Because the new developments in our knowledge of diabetes are stressed, the first several chapters deal with the chemistry and physiology of insulin and other hormones involved in carbohydrate, protein and fat metabolism. (Included are chapters on electron microscopy of the islets, intravascular fat and plasma insulin assay methods.) Roughly half the book is devoted to what one may call the "basic science" aspect of diabetes.

The second half is clinical in orientation with chapters on diagnosis, management, complications and prognosis, all very well done.

It is a commentary on changes in medical practice that only one of forty-eight chapters deals with the routine treatment of diabetes with insulin. As our knowledge of mechanisms increase it becomes more important to understand *why* and *how;* the *what*-to-do then follows logically. The chapters on oral antidiabetic agents and diet therapy (Marble) seem well balanced.

Although coverage is very wide, one would like to see a chapter on the social aspects of diabetes, i.e., employability, insurance risk, marriage counseling, psychologic factors, etc. This might balance the strong investigative and laboratory emphasis in the book.

This is, then, the most authoritative, current basic volume on diabetes. It is highly recommended.

S. O. W.

The Lifespan of Animals. Ciba Foundation Colloquia on Ageing. Vol. 5, edited by G. E. W. Wolstenholme and M. O'Conner. Little, Brown & Company, Boston, 1959, pp. 324, $9.50.

At this symposium fourteen papers were presented and discussed by twenty-two continental and English and five American investigators. Six papers and a general discussion of the rather haphazard collection of topics deal with longevity of man, horses, cattle and rats and eight papers with that of birds, insects and fishes. There is also an index of subjects dealt with and of authors participating in and referred to in this and the four previous colloquia on aging. Of some general interest are the papers by (1) B. Benjamin on actuarial aspects of human lifespans in which the advantages of some statistical methods are discussed, and (2) G. A. Sacher in which an attempt has been made to correlate brain and body weight to the lifespan of mammals. In the paper by E. Jalavisto, concerned with parental age effect on longevity of man, it is concluded that advanced maternal age has no effect on longevity of the progeny, once the latter have reached the age of fifteen years, although juvenile mortality during the first year of life seems to be slightly increased when the mothers were older than thirty-five years. Paternal age did not influence survival of the offspring. The correlation of onset of disease and lifespan is discussed in the paper by Simms and collaborators, and Scheidegger reviews arteriosclerosis in birds.

The presentation of the papers and the discussions are rather subjective, and there is an unfortunate disregard of important contributions to the field. Instead, as is customary for this type of meetings, the participants restate their views without adding significant new material. The personal exchange of views made possible by these symposia is certainly of value to the participants, but the printed version of the colloquium would be of greater value as a source of reference if the earlier work in the field had been quoted or referred to. Considering the small size of the booklet, the price seems rather high. M. SILBERBERG

World Review of Nutrition and Dietetics, edited by Geoffrey H. Bourne. J. B. Lippincott Company, Philadelphia, 1960, pp. 272, $12.00.

This is a unique book. It consists of ten chapters which, in the words of the editor, are discursive reviews that are critical evaluations of special fields. It is international in scope with articles by French, Japanese, Central American, British and American workers. The objective is "to describe the nutritional status of peoples under different geographic, climatic and economic conditions, and to examine all available knowledge in order to find solutions wherever nutrition problems exist."

The first of what will be a series of reviews contains a concise history of nutrition by E. V. McCollum. Other topics range from the biosynthesis of vitamin C to the use of isotopes in nutrition research, from kwashiorkor to neoplasia, with chapters on specific endocrine, embryologic and hematologic topics. A curious but fascinating chapter by Suzuki is a review of the basal metabolism among the Japanese. At first glance this topic would seem to be of limited interest but some data therein are of wide significance. For example, the basal metabolic rate of the Japanese fell about 10 per cent during World War II and is attributed to the generalized food shortage. However despite an improved postwar food situation, it took one to two years for recovery of the basal metabolic rate to take place. As the author states, it is quite conceivable that long-term undernutrition has a significant influence on the living organism.

All those seriously interested in nutrition will find this volume interesting and pertinent. Even scientists not working directly in the field will find this review informative and stimulating. S. O. W.

Abstracts of Current Literature

NEWER DIETARY STUDIES

The survival of men under adverse environmental conditions has been of considerable military significance. Among the most important factors is a compact, easily preserved source of energy. Although pemmican, as a food, consists of protein and fat it is capable of maintaining metabolism over periods of a week without deleterious effect.

Some Metabolic Effects of a High-Fat, High-Protein Diet During Semistarvation Under Winter Field Conditions. H. F. Drury, D. A. Vaughan and J. P. Hannon. *J. Nutrition*, 67: 85, 1959.

The use of survival rations high in fat and protein, with little or no carbohydrate, such as is found in pemmican, is of practical importance, especially under Arctic conditions. Heretofore when starvation studies have been made, it has generally been the fat and protein portions of the diet that were low, with carbohydrate predominating. On a high fat, high protein, low carbohydrate diet a condition of ketosis may be expected and feared.

This study was undertaken to determine whether men can function adequately for a reasonable length of time under realistic survival conditions on a diet consisting almost entirely of pemmican. A further purpose was to measure some of the effects of a small sugar supplement to the basic pemmican diet. Ten healthy adult men were used in the experiment. They were outdoors or in unheated quarters all but two hours a day. Temperatures ranged from + 14° to −55°F. Each man received 1,000 calories a day for nine days. The "sugar" groups received 40 gm. per day substituted isocalorically for pemmican.

Subjects receiving the sugar-supplemented diet exhibited significantly higher blood sugar levels and somewhat less ketone body excretion; nitrogen balances were not affected. Either diet, pemmican with or without sugar, "was deemed adequate for most survival situations faced by air-crews in the Arctic."

FRANK E. RICE

Some Biochemical Effects of Restricted Diets During Successive Field Trials in Winter. D. A. Vaughan, H. F. Drury, J. P. Hannon, L. N. Vaughan and A. M. Larson. *J. Nutrition*, 67: 99, 1959.

A succession of experiments and observations by these investigators with human subjects on restricted diets have demonstrated a greater than expected adaptation to low carbohydrate, low calorie diets, with persistence of adaptation following "recovery" periods of *ad libitum* feeding. This study is related to the research reported by Drury, Vaughan and Hannon. (See previous abstract.) The same environmental conditions, living arrangements, etc., reported therein, were employed in this study. Again, pemmican was fed at the rate of 1,000 calories a day. Three diets were used, pemmican supplemented isocalorically by 0, 40 and 80 gm. sugar per day. Each group of men, after five-day feeding periods on one of the three diets (period I) was allowed a "recovery" period of one week on a mixed diet *ad libitum*. Then followed a second feeding period (period II) in which each group received either more or less sugar than in the first period.

Eighty gram sugar supplements compared with the 40 gm. level had little if any additional influence on fasting blood sugar, nitrogen balance and ketonuria. Responses in period II differed both quantitatively and qualitatively from those observed in period I. The authors point out the need for caution in interpreting the responses from field studies in which recovery intervals between dietary regimes are no more than a week. Individuals who have apparently recovered from a

stress retain a latent capacity for the effective management of a subsequent stress of the same type.

FRANK E. RICE

A Nutrition Survey of the Armed Forces of the Republic of Korea. The Interdepartmental Committee on Nutrition for National Defense. R. R. Williams *et al. J. Nutrition*, 68: 1, 1959.

This is an eighty-page report on a survey conducted during June and July 1956 designed "to assess the current nutritional status of the troops." The survey included the assembling of information on agriculture and collection of dietary information supported by food analyses. This report describes the procedures used and major findings.

The diet of the armed forces in Korea is a simplified version of the national diet adjusted to mass feeding, predominantly rice with about 81 per cent of the caloric intake made up of cereals, 5 per cent of fish, meat, eggs and milk, 4 per cent of oil seed fats, some fruit, nuts, vegetables and sugar. Undermilled rice was systematically used by the military establishments. This was not true so extensively in the civilian population. Among other factors of importance in contributing to the better diet of the military was the effective use of soybeans and dried fish. The total protein intake/man/day averaged 117.2 gm., of which 22 gm. was from animal (or marine) sources. Milk and fresh meat were not available in liberal supply.

Examinations of 1,514 soldiers revealed limited evidence of serious deficiency disease, even though the dietary studies indicated suboptimal intakes of riboflavin, vitamin A and carotene. Active angular lesions of the mouth were observed in 13.8 per cent of the troops and 16.3 per cent excreted less than 30 μg. of riboflavin per six-hour urine specimen. The fact that physical and biochemical findings did not show marked indications of vitamin A deficiency is presumed to be due to seasonal high intakes of green vegetables from the military gardens. The overall dietary, biochemical and clinical results do not suggest that protein malnutrition is a widespread problem in the Korean armed forces.

FRANK E. RICE

Nutritional studies designed to evaluate the influence of dietary patterns upon the medical status of population groups must include an analysis of concurrent infections or infestations among the subjects. The conditioning effect of disease upon deficiency states is widely recognized and frequently represents an important contributory factor in making a marginal diet inadequate.

A Clinical Description of the Main Nutritional Disorders Encountered in the Salisbury Native Hospital in a Series of Fifty-Four Consecutive Cases Studied in 1954. J. Miller-Cranko and M. Gelfand. *Central African J. Med.*, 4: 16, 1958.

This article is a clinical description of the features of deficiency disease affecting the African adult in Mashonaland. The majority of the males are employed in industry in and around the periphery of the city of Salisbury. The diet consists of a good supply of maize and meat (about two and a half pounds per week). Fresh vegetables are not popular and beans and nuts are often preferred. Almost all subjects are affected with bilharziasis and almost all have malaria. A fair proportion have hookworm. About 15 or 20 per cent have a positive blood Wassermann reaction. These are relatively more primitive tribal subjects, and many prefer a "witch doctor." The climate is that of south central Africa.

Of fifty-four persons studied during 1954, thirteen (24 per cent) had vitamin A deficiency, but twenty-two (41 per cent) had pellagra. There was only one case of pure scurvy. In twelve cases both vitamin A and vitamin B complex deficiency was present, and in six cases the "pellagroid type" of skin lesion was noted. Among the striking clinical features were cheilosis (48 per cent), dryssebacea (50 per cent) pigmentation of the arms (48 per cent) hypertrophic pigmented follicles of the legs (44 per cent) and thin and wasted features (24 per cent).

S. O. WAIFE

Dietary Habits in Population Groups in Guatemala. IX. Santa Catarina Barahona. M. Flores, Z. Flores and B. Meneses. *Arch. Venezol. nutricion*, 8: 57, 1957.

A dietetic survey has been made in twenty-four families in rural areas of Guatemala. Individual data on all children included in the study were obtained. The results showed that the amounts of vitamin A, riboflavin and ascorbic acid consumed were low as compared to the recommended levels, adjusted to the weights of the studied group. Ingestion of calories, niacin and protein was just adequate, while that of calcium and thiamin was relatively high.

In the children, ingestion of all the nutrients was inferior to the recommended allowances, with the exception of iron. The quantity of food consumed by the children was very low, resulting in intakes of vitamin A and riboflavin below 50 per cent of the recommended level. Consumption of animal proteins is very low, only 5 to 6 per cent of the total protein. Corn is the main protein source.

AUTHOR

The calculation of the sodium content of the diet has been known for many years to result in substantial errors when standard tables are employed. For accuracy in metabolic determinations, duplicate portions of consumed foods must be subjected to biochemical analysis of electrolyte content.

Error in the Provision of Diets of Known Electrolyte Content. C. T. G. Flear, P. Huges, and I. McCellan. *Brit. J. Nutrition*, 13: 54, 1959.

Much metabolic work is dependent upon the feeding of diets of known composition. In addition, the clinician wants to prescribe a diet of known electrolyte content and expects the quantitative aspects to be accurate. In this paper, a study is described in which two diets

prepared for balance studies and diets provided during routine management of patients were analyzed, and the values found for sodium, potassium and chloride compared with those found by calculation from tables of food composition. Diets were prepared over a period of six weeks and on any one day they were prepared from the same cuts of meat, same loaf of bread, etc. All diets were prepared by the same person.

On the average, the electrolyte content as determined by calculation gave a 13.5 per cent underestimate for sodium, a 10.4 overestimate for potassium and a 1.4-per cent overestimate for chloride. Variations of triplicate examples were 4 per cent or less. It is particularly interesting that the low sodium diet as actually prepared contained more sodium than that intended.

S. O. WAIFE

There is little information available concerning the influence of diet upon the eyes outside the field of deficiency states. The effect of a diet more liberal in protein, than that fed control subjects, in improving myopia requires confirmation.

Dietary Treatment of Myopia in Children. P. A. Gardiner. *Lancet*, 1: 1152, 1958.

The author has shown in previous reports that myopic children are more fussy about their food than other children and in particular eat less animal protein. In this study an attempt has been made to correct the defect by correcting the diet. Two groups of school children were the subjects of this study. They were roughly comparable in terms of myopia and in health and social status. Those with congenital myopia were excluded. One group was given a diet in which 10 per cent of the calories consisted of animal protein, the calorie intake itself being left to the child's natural appetite; the other group had no dietary advice. The two groups were studied for a year.

The rate of visual deterioration was much less in the children treated and, after the age of eight, there was virtually no deterioration in this group. Some older children who took the greatest quantities of animal protein actually showed an improvement. The possible reason for the benefit is discussed; it is still obscure.

F. E. HYTTEN

Dietary studies on experimental animals have proved of considerable value in determining the biologic value of foods and the role of micronutrients in cellular metabolism. However, one must be cautious in assigning clinical significance to observations on animal diets.

Comparative Performances of Baby Pigs Fed Infant and Baby Pig Diets. F. Diaz, V. C. Speer, P. G. Homeyer, V. W. Hays and D. V. Catron. *J. Nutrition*, 68: 131, 1959.

It has been amply demonstrated that the rat is not a suitable subject for dietary studies in which human milk and products resembling it in composition are used. This is because of the animals' low tolerance for lactose, which is high in human milk and in most infant foods. The pig can utilize lactose very effectively. This research was undertaken to study the performance of baby pigs fed commercially prepared infant foods in comparison with standard baby pig diets, the ultimate objective being to determine whether or not the baby pig could be used as a test animal for evaluating human infant diets.

In these experiments 136 crossbred pigs were used. In one experiment, the average age was 11.1 days and average weight 5.8 pounds; in the other 7.1 days and 6.3 pounds. Previously tested baby pig diets were prepared for comparison, which contained dried skim milk as a base with added fats and necessary nutrients. Five milk-base commercial infant feeding products were fed in the experiments, and two soybean products. In some cases the feed was offered in dry form, in others the products were uniformly reconstituted with water to a 20 calories/ounce dilution. The principal differences in composition of the reconstituted fluids were in content of protein and fat. After reconstituting to the 20 calories/ounce basis, protein and fat percentages were respectively as follows: in the five milk-base foods—A 3.4 and 2.7 per cent; B 2.7 and 2.8 per cent; C 2.7 and 2.8 per cent; D 1.7 and 3.4 per cent; E 1.5 and 3.5 per cent; in the two soybean infant foods —F 3.2 and 2.6 per cent; G 3.1 and 4.0 per cent.

Infant formulas A, B and C performed well, in general, equal to or better than the standard pig diets. Formulas D and E produced consistently low rates of gain and high feed requirement per pound of gain. Performance on diets F and G (soybean base) was unsatisfactory. This is attributed mainly to the (previously observed) inability of the pig to utilize soya protein adequately. It is concluded that baby pigs grow satisfactorily on infant (cow's milk) formulas when the protein level is 2.7 to 3.4 per cent, and less satisfactorily at levels of 1.7 and 1.6 per cent protein.

"The baby pig shows promise as a test animal in biologically evaluating human infant diets, however, these data need to be corroborated with carefully controlled clinical studies and general observations with the human infant."

FRANK E. RICE

Effect of Dietary Level of Fat and Type of Carbohydrate on Growth and Food Intake. A. Yoshida, A. E. Harper and C. A. Elvehjem. *J. Nutrition*, 66: 217, 1958.

The purpose of this study was to determine the interrelationships that might exist among dietary levels of fat, types of dietary carbohydrate and of fat, growth rate, food intake, and the volume and moisture content of stomach contents. Earlier experiments had indicated that the feeding of diets containing low molecular weight carbohydrates to rats resulted in lower food intakes and growth rates than the feeding of isocaloric diets in which the carbohydrates were of high molecular weight. The influence of osmotic pressure of stomach contents was suspected.

Oleomargarine was used in all diets as the main source of fat unless otherwise stated. Other fats studied were butter, lard, olive oil and hydrogenated coconut oil. All diets contained 1 per cent corn oil, including the "negative control group." The carbohydrates tested were sucrose, cerelose and dextrin. Comparative diets were isocaloric, also isonitrogeneous (at two levels). Feeding was *ad libitum*.

In the experiments with fats it was observed that in all cases 30 per cent fat in the diet caused more rapid weight gains than the negative controls. There were little differences between the various types of fats fed, although the data seem to show a tendency toward greater relative gains in the two-week periods when butter was the fat incorporated in the diet. The relative gains per calorie intake were about the same for all rats. There was a high positive correlation between growth and caloric intake regardless of the carbohydrate source or the dietary level of fat in experiments in which the protein to calorie ratio was kept constant.

The groups fed dextrin increased in weight faster than those fed sucrose or cerelose at the 1 per cent and 10 per cent fat levels. There were no differences at the 30 per cent fat level. Stomach contents of rats one and a half hours after ingestion of 5 gm. of ration averaged 9.5 gm. in the animals on the sucrose diet; 6.9 gm. was the average for the groups fed dextrin. There was no evidence that fat was emptied from the stomach more slowly than either carbohydrate or protein; however, the authors recommend further study of this point.

"It is suggested that, when a substantial part of a low-molecular weight carbohydrate in a diet is replaced by fat or dextrin, the osmotic effect of the diet is reduced sufficiently to permit young rats to consume a greater quantity of diet and, hence, gain weight more rapidly during the early stages of growth." FRANK E. RICE

ITEMS OF GENERAL INTEREST

Gastroscopic Findings in Pellagra. Their Correlation with the Clinical Manifestations Before and After Treatment. M. Salib. *Gastroenterology*, 36: 816, 1959.

The author examined forty patients with typical pellagra. Eighty per cent of the patients had gastrointestinal symptoms: most commonly, epigastric discomfort after meals, diarrhea, and anorexia or nausea. Involvement of the nervous system was common—60 per cent having polyneuritis, 25 per cent lateral sclerosis and 10 per cent dementia. All were moderately or markedly anemic.

Of the forty patients, twenty-eight were found to have gastric atrophy on gastroscopic examination. In the group with gastric atrophy, twenty had diminished or absent gastric acid secretion. Eight patients with gastric atrophy were treated with injections of nicotinic acid (300 mg.), thiamine (100 mg.) and riboflavin (10 mg.) for fifteen days, together with a nutritious diet. All showed reversal of gastric atrophy to normal, along with disappearance of skin rash, gastrointestinal symptoms, anemia and achlorhydria. However, neurologic manifestations persisted. Another group of eight patients failed to respond to therapy with vitamin B_{12}.

Although the observations strongly suggest that the gastric atrophy and diminished gastric secretion were due to pellagra, other nutritional deficiencies may have played a part. These data are valuable since there is very little information available on the effects of dietary deficiency on gastric mucosal integrity and gastric acid secretion. J. B. HAMMOND

Effect of Ionizing Radiation on the Allergenicity of Milk Protein. H. F. Kraybill, R. O. Linder, M. S. Read, T. M. Shaw and G. J. Isaac. *J. Dairy Sc.*, 42: 581, 1959.

The objective of this investigation was to study the effect of ionizing radiation on the allergenicity of milk protein as measured by gross anaphylaxis (guinea pigs), and by the Schulz-Dale uterine strip technics. Four batches of raw skim milk were radiated at 0.465, 2.79, 5.58 and 9.30 megarads at the Idaho Falls reactor. Another sample of whole milk processed by radiation distillation at the Massachusetts Institute of Technology, using Co^{60}, was also tested. Reductions in allergenicity in skim milk at the higher levels of radiation were observed to be of sufficient magnitude to be of "clinical importance." The radiation distillation samples could not safely be compared without further experimentation to determine whether some indirectly reactive compounds may have been removed in the distillation process. The possible protective action of the fat also is an unknown factor. FRANK E. RICE

Incidence of Familial Hyperlipemia. K. Hirschhorn, R. Hirschhorn, M. Fraccaro and J. A. Böök. *Science*, 129: 716, 1959.

Familial hyperlipemia is an inherited disease associated with early onset of coronary atherosclerosis. It is thought to be due to a single gene difference causing a defect in the lipemia-clearing system. The homozygous condition is characterized by hepatosplenomegaly, abdominal crisis, milky serum, childhood atherosclerosis and xanthomatosis. In this study, a survey of the student population in Sweden is reported. Samples were obtained from 998 consecutive students at a Swedish university. The samples were examined for optical density, and where there was a density above a certain level, the serum was analyzed for lipids. Thirty-six showed a significant optical density; of these, sixteen demonstrated a marked delay in clearing of neutral fat from the serum, and four had a borderline delay. Five students were found to have primary hypercholesterolemia and eight were completely normal.

The incidence of familial hyperlipemia in an apparently normal northern European population was close to 3 per cent.

Three persons had an elevated optical density of the serum despite the ability to clear ingested fat normally. A study of the serum in one case showed that the mate-

rial was a form of lipid and may represent an abnormal chylomicron. S. O. WAIFE

Postnatal Vascular Growth and Remodelling in the Pathogenesis of Arterial Lesions. As seen from a study of Micro-Anatomical and Histochemical Changes in Vascular Elastic Membranes Injured by Odoratus Intoxication. T. H. Gillman and M. Hathorn *Schweiz. Ztschr. Allg. Path.*, 22: 62, 1959.

Male rats of the Wistar strain were fed a diet composed of 50 per cent ground sweet pea seeds and 50 per cent stock ration; an equal number of animals serving as controls were fed the stock diet only. At the age of 83 and 113 days, respectively, the diets of half of the rats of either group were enriched with 10 per cent lard and 2 per cent cholesterol until the end of the experiment. After fifty-two and fifty-three days, aortic ruptures occurred in the animals fed the lathyrus factor. However, the older the animals at the beginning of the experiment, the less frequent were these lesions. The well known skelatal changes also were more severe and more frequent the younger the rat, when fed the lathyrus seeds. The main change of the ground substance in the aortic lesions consisted of progressively increasing metachromasia as well as ferrocyanide-positive polymucosaccharides in the peri- and intermembranous layers, alterations in the amount and distribution of PAS-positive material, deposition of granular elastic-positive material about and between the elastic membranes, increased waviness and merging of the latter and inconsistent alterations in the adjacent reticulum. Adult rats were practically resistant to the toxicity of the lathyrus seeds. The lathyrus-induced lesions are thought to be due to depressed synthesis of new collagen-like material about the membranes rather than to lysis of pre-existing ones. M. SILBERBERG

Simultaneous Flux of Potassium Into and Out of the Dog Intestine. E. Y. Berger, G. Kanzaki and J. M. Steele. *Am. J. Physiol.*, 196: 1270, 1959.

Potassium moves across the intestinal mucosa from the lumenal surface to the blood simultaneously with a stream in the opposite direction from blood to lumen. The net result of these two flows determines the amount of potassium appearing in the intestinal lumen. These two flows were measured across isolated loops of intestine in the dog with the use of radiopotassium. For a 20 cm. length of intestine, potassium enters the lumen at 0.8 to 6.5 μEq. per minute, and simultaneously leaves the lumen at 0.7 to 5.2 μEq. per minute.

AUTHORS

I^{131}-Labeled Protein and Fat Meals in Patients with Chronic Pancreatitis. A. Polachek, C. B. Cope, R. F. Williard and F. W. Barnes, Jr. *Gastroenterology*, 37: 38, 1959.

In order to determine the relative value of radioiodinated protein and of fat meals in detecting malabsorption in patients with chronic pancreatitis, fourteen patients with this condition were studied by both methods. Plasma radioactivity, at hourly intervals, and fecal and urinary radioactivity for forty-eight and seventy-two hour periods were determined. Data by one method only were obtained in ten additional patients with chronic pancreatitis.

In sixteen normal control subjects, the mean maximal plasma I^{131} absorption after a protein meal was 13.5 per cent (standard deviation 2.3 per cent). In eight patients with mild chronic pancreatitis, the corresponding value was 13.8 per cent. In four patients with severe chronic pancreatitis, as evidenced by pancreatic calcification, the mean maximal plasma I^{131} absorption was 10.6 per cent; and in seven patients in whom chronic pancreatitis was associated with pancreatic calcification and diabetes mellitus the corresponding value was 7.8 per cent.

In the sixteen normal control subjects, the mean maximal plasma I^{131} absorption after a fat meal was 13.7 per cent (standard deviation 2.6 per cent). In the group with pancreatitis alone, the corresponding value was 12.4 per cent; in the group with pancreatitis and calcification, it was 4.9 per cent; and in the group with calcification and diabetes mellitus, it was 3.1 per cent.

It was concluded that the radioiodine-labeled triolein studies provided the greatest differentiation between patients with the more severe forms of pancreatitis, as compared with the protein meal studies. Neither type of test gave abnormal results in the patient with mild, uncomplicated pancreatitis.

Fecal excretion of the tagged fat meal usually was related reciprocally to the plasma I^{131} levels. Fecal excretion of the protein meal was seldom abnormal. The determination of the radioiodine content of urine samples following either protein or fat meals was of no practical value. J. B. HAMMOND